EFFECTS OF LANDSCAPE CHARACTERISTICS ON FRAGMENTED WETLAND PLANT COMMUNITIES AND EXPERIMENTAL METAPOPULATIONS OF *Arabidopsis thaliana*

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Chapter 1

General Introduction

GENERAL INTRODUCTION

Habitat fragmentation is a landscape-level process of subdivision of once large, connected habitat areas into smaller and more isolated fragments (McGarigal & McComb 1995) and has been recognized as a major threat to global biodiversity (Noss 1983; Wilcox & Murphy 1985; Saunders et al., 1991).

Anthropogenically induced changes in landscape elements often create islands of natural habitat embedded in an unsuitable matrix (Groom & Schumaker 1993; Hanski 1999; Cook *et al.*, 2002). In many plant species, populations are patchily distributed, and therefore the regional survival of these plant species is influenced by the habitat configuration (Saunders *et al.*, 1991; Hanski & Ovaskainen 2000; Ney-Nifle & Mangel 2000). The distribution of suitable habitat and the dispersal abilities of the organism effectively connect landscape elements (Gardner et al., 1987; Kindlmann et al., 2005; O'Neill et al., 1988; With & Crist 1995).

To date, many researchers have focused on the effects of patch size on species richness, and the effects of habitat isolation, inter-patch distance and landscape connectivity on the behaviour of a single or few species (e.g. MacArthur & Wilson 1967, Taylor et al., 1993, Fahrig & Merriam 1994, Hanski 1999). However, there is a lack of studies on the relationship between landscape patterns and biodiversity (Hersperger 2006) and on change in species compositions and species migration patterns and extinction risks in fragmented landscapes under environmental change (Jump & Penuelas 2005).

In my Ph.D. thesis I conducted both observational and experimental studies on the effects of landscape patterns (habitat fragmentation and spatial structure) and landscape dynamics (changes in landscape patterns through time) on plant communities (their relative abundance of species and their spatial/temporal relationship to each other).

Evolutionary and ecological processes in spatially structured landscapes were investigated at different scales from natural landscapes to experimental microcosms and involved observation of large-scale population processes as well as experimentally manipulated short-lived metapopulations.

Habitat fragmentation observational studies vs. experimental studies

There are many studies on the ecological effects of spatial structure and habitat fragmentation on different species (e.g. Saunders et al., 1991; Debinski & Holt 2000; McGarical & Cushman 2002) using a variety of approaches, including field observational and experimental approaches, as well as spatial models. Each of these approaches has its advantages and disadvantages. However, to date, there are many more observational studies than experimental studies of fragmented landscapes, and few researchers have created experimentally fragmented landscapes (but see Gonzalez et al., 1998; Fox 2007). Because, fragmentation effects often operate at large spatial and temporal scales, it is often impossible to manipulate entire landscapes. To avoid the limitations associated with studying large-scale landscape, the use of Experimental Model Systems (EMS) has been advocated (Lawton 1995). However, such EMS may lack generality. They are species, scale, and design-specific and sometimes too simplified to allow understanding the complicated systems in the real world. On the other hand, in nature, one of the difficulties in studying the effects of habitat fragmentation is that at the landscape scale, it is very difficult to find replicates and nearly impossible to find sets of similar habitat arrangement for comparison. Artificially created metapopulations may help to overcome this difficulty and provide powerful tools to evaluate our understanding of evolutionary and ecological processes in spatially structured systems.

This dissertation

Observational study: wetlands project

In the wetlands project, changes in the biodiversity of fen meadows in NE Switzerland over the last 10 years, have been studied. New data was collected by myself and colleagues and compared to an existing data set collected ten years ago.

This Ph.D. thesis focuses on the spatial aspects of biodiversity: 1) The decay of species compositional similarity between sites (a measure related to ß-diversity), 2) landscape spatial patterns and its effect on ecological process (plant species diversity and composition), and the possible temporal change of the effects in these protected wetlands within the last 10 years.

However, in parallel to this thesis, Ariel Bergamini focuses on the effect of management type on biodiversity of theses same fen meadows (for more details: see Bergamini et al., 2009), and Hossein Moradi, in his Ph.D. dissertation, addresses the effects of functional traits and different plant species-group responses (colonization and extinction rates) to climate change (for more details: see Moradi et al., submitted). I helped to collect the data for both of these additional studies.

Experimental study: Arabidopsis project

To assess the consequences of habitat fragmentation and landscape dynamics on plant populations, we artificially created islands of suitable habitat embedded in an unsuitable matrix to simulate islands of natural habitats in nature (Hanski 1999, Cook et al., 2002) using *Arabidopsis thaliana* as a model species. We have used two *Arabidopsis* genotypes that show an almost 4-fold difference in their mean seed weight: Landsberg *erecta* (L*er*) and Cape verde Islands (Cvi) in an experimental population of recombinant inbred lines (RILs) derived from the two genotypes. We investigated in this experiment selection on dispersal abilities (seed size and plant height) of

Arabidopsis thaliana over five generations in landscapes differing in the degrees of habitat fragmentation and disturbance rate of patches.

Outline of this dissertation

Following this introductory chapter, the thesis is composed of 4 chapters: the first two describe vegetation composition patterns and recent vegetation change in fen meadows of the foothills of the Swiss Alps by landscape-scale processes (giving a total of 180 plots distributed over the 36 fens); and the last two chapters describe the experimental study in which we manipulate landscape characteristics, degree of fragmentation and the rate of patch disturbance, for populations of the annual plant *Arabidopsis thaliana.* In our experiment we assembled a group of inbred lines which differed in their dispersal traits and monitored selection among the different lines in 180 habitat patches.

- **Chapter 2** focuses on changes in species compositional similarity of fen meadows within the last 10 years, and asks whether the pattern of similarity decays over geographical distance differed 1) between altitudinal levels (800–1000, 1000–1200, 1200–1400 m a.s.l.), 2) between management regimes (mown vs. grazed), 3) between taxonomic groups (vascular plants vs. bryophytes), and 4) between specialist and generalist plant species and whether patterns showed temporal consistency by comparing similarity–distance relationships between the two census periods (1995/97- 2005/06).

- **Chapter 3** explores the relationship between landscape spatial characteristics and fen species diversity over time. Specifically, we tested how fen habitat characteristics (patch size, distance between fens, and number of neighbouring fens), surrounding habitat types, and potential

landscape permeability may influence plant and bryophyte species diversity and the possible temporal change of the effects in these protected wetlands within the last 10 years.

- **Chapter 4** considers the effect of experimental small-scale landscape manipulations on *Arabidopsis* population characteristics which we did not deliberately manipulate such as density of seedlings, survival of plants to adulthood and total biomass. I ask how patch size and disturbance rate affect population density, biomass and survival rates.

- **Chapter 5** focuses on the outcome of selection on dispersal abilities and plant traits (seed size and plant height) of *Arabidopsis thaliana* and asks which characteristics of the plants (height and seed mass) are selected under different spatial/ temporal habitat fragmentation?

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Chapter 2

Homogenization of plant communities in fen meadows over the last ten years in Switzerland

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ABSTRACT

Similarity in species composition between sites typically decreases with geographical distance due to a decrease in environmental similarity or due to dispersal limitation. We studied the decay of similarity with distance between plant communities (a measure related to ß-diversity) among 36 species-rich, pre-Alpine fen meadows distributed across north-eastern Switzerland both in 1995/97 and again in 2005/06. We tested whether the pattern of similarity decay over geographical distance differed 1) between altitudinal levels (800–1000, 1000–1200, 1200–1400 m a.s.l.), 2) between management regimes (mown vs. grazed), 3) between taxonomic groups (vascular plants vs. bryophytes), 4) between specialist and generalist plant species, and 5) we tested whether patterns showed temporal consistency by comparing similarity–distance relationships between the two census periods. Geographical distance between pairs of sites was defined as edge-to-edge distance and similarity in species composition was measured with the Jaccard index. These distance data were analyzed with Mantel tests and multiple regression models. Compositional similarity among vascular-plant communities decayed significantly with geographical distance only at higher altitudes (in 1995/97: both at 1000–1200 m and 1200–1400 m, in 2005/06: only at 1200–1400 m), but not at the lowest altitude. For grazed fens, the rate of decay was significant in 1995/97, but not anymore in 2005/06. Furthermore, the decay of similarity over geographical distance in generalist vascular plants and bryophytes was significant in the first census in 1995/97, but not in 2005/06. These results indicate that geographically structured ß-diversity among sites has decreased over the past 10 years especially so at lower altitudes. Results of complementary studies suggest that this homogenization is due to an increased occurrence of common generalist species and a concomitant decrease of fen specialist species. Therefore the observed change in geographically structured ß-diversity presents a

concern for the conservation of these fen habitats that originally hosted a suite of specialised and endangered species.

Keywords: altitude, ß-diversity, distance decay, Jaccard similarity index, species composition, vascular plants, bryophytes

INTRODUCTION

Decay of similarity in species composition with increasing geographical distance between sites is a well-known phenomenon (Nekola & White 1999; Poulin 2003; Soininen *et al.*, 2007; Morlon *et al.*, 2008). Such a decay of similarity over geographical distance or among-community richness within a geographic region is sometimes referred to as ß-diversity (Harrison *et al.*, 1992). Studying patterns of species compositional similarity along spatial or environmental gradients has become an important step in regional biodiversity planning (Loreau 2000; Passy & Blanchet 2007).

The geographical distance-related decay of community similarity may result from a decrease in environmental similarity between sites or from dispersal limitations (Nekola & White 1999). Hubbell's neutral theory (Hubbell 2001) explains the distance-related decay in similarity mainly by limited dispersal abilities of species between sites. Based on neutral theory, compositional similarity is not affected by environmental heterogeneity, but rather by ecological drift, random dispersal and random speciation (Hubbell 2001). The alternative functional interpretation of a distance-related decay in similarity is that sites that are more distant are likely to differ more strongly in environmental conditions than close sites and therefore harbour different species adapted to the different environmental conditions of the different sites (Bell *et al.*, 2006). Geographical distance–similarity relationships may be non-linear (Nekola & Brown 2007) and vary between different types of habitats (Tuomisto et al., 2003; Novotny et al., 2007), different groups of organisms (Poulin 2003; Oliva & Gonzalez 2005; Steinitz et al., 2005, 2006), different geographical gradients (Kerr & Packer 1997) and scales (Wohlgemuth 1998; Deutschewitz et al., 2003; Soininen *et al.*, 2007).

Biotic homogenization between sites can occur as a consequence of change towards a common land-use type, homogenizing effects of pollution or climate change and spread of

generalists and invaders (Kadmon & Pulliam 1993; Cottenie 2005; Olden & Rooney 2006; Rooney *et al.*, 2007; Vellend et al., 2007). Recent studies showed that biotic homogenization occurred in the last fifty years both in terrestrial and aquatic systems (Rahel 2000; McKinney 2004; Rooney *et al.*, 2004; Taylor 2004; Soininen *et al.*, 2007). For example, Radomski & Goeman (1995) showed an increase in fish compositional similarity over a 43-years period in 62 lakes in Minnesota or Rooney et al. (2004) demonstrated floral homogenization in 62 upland-forest plant communities between 1950 and 2000 in Wisconsin.

We studied the rate of distance-related decay of biological similarity in an archipelago of 36 species-rich fen meadows in the foothills of the Swiss Alps (800–1400 m a.s.l.). Prealpine fen-meadows harbour a species rich but specialized type of plant community adapted to the nutrient-poor and moist site conditions (Dietl 1975).

We tested how geographical distance, altitude (three altitudinal classes) and management type (grazing or mowing) affected the similarity of vascular plant and bryophyte species-compositions between fens and whether there was a trend towards biotic homogenization within the last 10 years.

METHODS

Field sites

Our study region is located in the Swiss pre-Alps and covers an area of approximately 3500 km^2 in north-eastern Switzerland (Table 1). We surveyed montane calcareous fen meadows of the *Caricion davallianae* alliance (Ellenberg 1996) that are of high conservation concern due to their high biodiversity (Peintinger et al. 2003, Wettstein & Schmid 1999), occurrence of many endangered species and recent reduction in area (Klaus 2007). In total, 36 sites were randomly selected from more than 300 fens of at least 1 ha listed in a national inventory (BUWAL 1990;

Bergamini et al., 2009). The selection was stratified into two management types (mown vs. grazed) and balanced for habitat area (see also Wettstein & Schmid 1999). The altitude of the chosen sites ranged from 800–1400 m a.s.l. (Table 1). At each of the three altitudinal levels, we chose 12 sites with a difference in elevation of up to 200 m within each level. The soil of our calcareous fen sites was nutrient-poor and base-rich (Bergamini *et al.*, 2001; Pauli *et al.*, 2002). Mean annual precipitation was 1500–2800 mm and bedrock mainly consisted of various calcareous sediments of tertiary and mesozoican age (Spicher 1972). The geographical distance among sites ranged between 0.55 and 71.7 km (Table 1).

Vegetation monitoring

In summer 1995 the presence/absence of vascular plants and in summer 1997 the presence/absence of bryophytes was recorded at all 36 sites. Each site was sampled using five randomly selected plots of 2 ×1 m, giving a total of 180 plots distributed over the 36 fens (see Bergamini et al., 2009, for a more detailed description of the monitoring process and the nomenclature used). The second survey took place in July and August 2005 (24 randomly selected fens out of the totally 36 fens) and July 2006 (12 fens). Shape and size of plots were identical to the first survey. For the designation of vascular plants with high habitat specificity (habitat specialists), we used all 25 species characteristic of the phytosociological order *Caricetalia davallianae* listed in BUWAL (1990). Because no similar list exists for bryophytes, we based our selection of bryophyte habitat specialists on Hajek (2006), but adjusted it according to own experience, which finally consisted of 19 bryophyte specialists (see Bergamini et al., 2009, for list of habitat specialists).

Mean α- and γ-diversity of vascular plant and bryophyte communities was calculated for fens in each of the three altitudinal classes, for fens under the same management type (mown vs.

grazed), and for habitat specialist and generalist species, for both surveys (Table 2). We calculated α-diversity as the cumulative number of recorded species in the five plots of each site (total 10m²) and γ-diversity as the cumulative number of species at all sites in each category. For instance, γ-diversity of vascular plants at 800–1000 m denotes the pooled number of vascular plants in all plots of the 12 sites, which where located at this altitudinal level.

Statistical Analysis

The linear distance between sites was calculated in ArcGIS 9.00 software (ESRI, 2004) based on edge-to-edge distances. Similarity in species composition between sites was calculated separately for different taxonomic groups (vascular plants vs. bryophytes) and ecological categories (specialists and generalists) of species using the Jaccard similarity index (Jaccard 1901; Mueller-Dombois & Ellenberg 1974; Legendre & Legendre 1998). The Jaccard similarity index was used because of its simplicity, widespread use, and reliance on presence/absence data (Nekola & White 1999). Both, presence/absence data as well as abundance data were available in this study, but we chose presence/absence data because it represents a more robust measure of community similarity than abundance based measures (Nekola & White1999). The Jaccard index ranges from zero to one and denotes the proportion of species shared by a pair of sites out of the total number of species present at these sites. If there are no species in common between two sites, the index equals zero. If two sites share exactly the same species, the index equals one. Matrices of geographical distances and Jaccard similarities were assembled 1) for all sites, 2) for sites within the same altitudinal level and, 3) for sites under the same management regime (Table 2).

Because the pair-wise similarities or distances within a matrix are not truly independent (Legendre & Legendre 1998), the significance of the correlation between similarity and distance was tested using a Mantel test (Mantel 1967; Jackson 1989; McCune 2002). Probabilities of type

I error were based on 999 permutations. *P*-values were calculated using Monte Carlo randomization of response variables in R 2.4.1 (R Development Core Team 2007).

Patterns of compositional similarity between sites were also analyzed with a repeated-measures analysis of variance to test the influence of the design factors: management type, altitudinal level, and time, species groups, and above all, to test the interactions of these factors on the similarity between each pair of sites (Appendix 1). For this repeated measures analysis, we compared similarity of sites which are located in the same altitudinal class and under the same management regimes.

Because of the symmetrical nature of the distance coefficient ($d_{ij}=d_{ji}$), the lower left portion of each matrix contained redundant values with the upper right portion of each matrix. After removing these redundant values and the main diagonal (self-distances, which equal zero) there were n(n-1)/2 values left per matrix. This number equaled the number of pairs of sites of which the species compositional similarity was related to geographical distance and the other study design factors. For example, for the mown fens at the lowest altitudinal level, there were 15 pairs of sites to be compared (6(6-1)/2). Because of space restriction and to enhance readability, just the first part of the ANOVA table is shown in Table 4 whereas all details of the repeated measures ANOVA are presented in Appendix. 1. The effect of altitudinal class was decomposed into a linear term ("altitude") and the remaining variation, i.e. the deviation from linearity ("deviation").

RESULTS

In total, 229 vascular plant species and 120 bryophyte species were recorded at the 36 study sites during the two census periods. The mean similarity between sites was 0.51(± 0.13) for vascular plants and 0.49 (± 0.17) for bryophytes, and did not change between the two censuses ($P> 0.1$).

Distance-related decay of compositional similarity at different altitudinal levels

The compositional similarity of vascular plant communities significantly decreased with geographical distance in 1995 at the higher altitudinal levels (1000-1200 and 1200-1400m), but not at the lowest altitudinal level (800–1000m; Table 3; Fig. 1 A). Ten years later, in 2005/06 a significant distance-related decay of similarity of vascular plant communities was observed only at the highest altitudinal level (1200–1400m) whereas only a trend was observed at the mid-altitudinal level and no decay at the lowest altitudinal level (Table 3). The compositional similarity of bryophyte communities only decayed significantly with geographical distance at the mid-altitudinal level (1000–1200m) in both censuses (Table 3).

Influence of management type

The distance-related decay of compositional similarity of vascular-plant communities was highly significant among grazed fens in 1995 (Table 3; Fig. 2a), but not anymore in 2005/06 (Table 3; Fig. 2b). In mown fens, the similarity decay for vascular plants with distance was marginally significant in 1995 and significant in 2005/06 (Table 3, Fig. 2).

Compositional similarity of bryophyte communities, tended to decline with distance for both management types in 1997, but not in 2005/06 (Table 3).

Difference between specialist and generalist species

In 1995/97, the compositional similarity between vascular plant and bryophyte communities was higher for specialist than for generalist species (Table 3, Fig. 3). Compositional similarity for specialists did not significantly decay over geographical distance over the whole study area in 1995/97 and in 2005/06 (Table 3). In contrast, compositional similarity for generalists declined

significantly (bryophytes) or marginally significant (vascular plants) over geographical distance in 1995/97, but not in 2005/06 (Table 3).

Interactions between factors influencing compositional similarity

Geographical distance and management type explained 33% and 12%, respectively of the total variation in compositional similarity between sites (Table 4). The remaining variance was explained by effects of altitudinal level and interactions between the factors mentioned above (Appendix 1). For example, there was a significant 3-way interaction between specialist/generalist species (vascular plants and bryophytes combined), altitudinal level and census time ($F= 6.15$, $P<0.01$, see Appendix 1): Diversity in species composition between sites declined particularly strongly with distance for generalist vascular plants at low altitudes**.**

DISCUSSION

Changes in compositional similarity over the 10–year observation period

Within ten years, we observed an increase in compositional similarity over geographic distance indicating ongoing homogenization in the study region. However, the pattern of increasing homogeneity and decreasing variation in species composition was not uniform across all altitudinal levels and depended on management type as well as on the taxonomic and ecological group studied. Compositional similarity of vascular-plant communities between sites generally declined with geographical distance, but at the lower altitudes this general trend was no longer present in the second survey. This homogenization of vegetation composition at lower altitudinal levels was mainly due to generalist species whose presence increased across the entire set of 36 sites (see Moradi et al., in prep, Bergamini et al., 2009). This trend was confirmed by the increase

in compositional similarity over geographic distance of bryophytes and vascular plants within the last ten years.

Biotic homogenization may occur via a variety of mechanisms (Olden & Rooney 2006). Species extinctions both at the α- and γ-diversity level as well as increased colonization among regions (γ-diversity) and/or sites (α-diversity) may lead to biotic homogenization among sites. Alpha and γ-diversity may increase due to easier dispersal (hence a more neutral process), or rather due to processes based on habitat change, i.e. changed environmental conditions permitting new and common species to invade sites. Besides such colonization effects on the observed homogenization in our pre-Alpine fens, extinction processes can also contribute to homogenization: in our fen meadows investigated, vascular plant specialists decreased both in α- and γ-diversity within the 10-year observation period (see Table 2; for more details see Bergamini et al, 2009). In contrast to specialist vascular plants, α- and γ-diversity of generalist vascular plant species increased over the 10-year observation period (Moradi et al, in prep).

For bryophyte species composition, decay of similarity over geographical distance was generally low in our study sites. The smaller size of bryophytes may allow persistence in micro-sites not available for vascular plants and bryophytes may have broader physiological tolerances and higher persistence rates because they can cycle rapidly between active and dormant states (Nekola & White 1999). In addition, many bryophytes are good dispersers (Miles & Longton 1992) and rely mainly on wind for dispersion of their spores, which contrasts with the various vectors used by vascular plants for their on average heavier seeds. However, also in bryophytes the distance-related decay of compositional similarity was less pronounced in 2005/06 than in 1995/97 and as for vascular plants, the generalist bryophytes were mainly responsible for the observed homogenization (see Table 3).

The underlying reasons for the indicated colonization and extinction processes among generalist and specialist species, respectively, leading to the homogenization of species compositions across geographical distance, may have been caused by a range of factors such as land-use changes in the surroundings, atmospheric nitrogen deposition or climate change (Bergamini et al., 2009; Moradi et al., in prep; Fakheran et al., in prep.). Some recent studies show an upwards shift of plant species due to global warming leading to increased species numbers (Grabherr et al., 1994; Kullman 2002; Parmesan & Yohe 2003; Walther et al., 2005), probably also involving many generalist species.

Difference in distance-related similarity decay between altitudinal levels

Similarity was decreasing faster over geographical distance at high than at low altitudes, indicating that fens at higher altitudes differ more with increasing distance. However, this effect was weaker in the second survey. It is known that the absence of successful dispersal among patches can contribute to higher values of ß-diversity (Chase 2003) and therefore to a higher rate of decay of similarity over geographical distance. At higher altitudinal levels, the pool of available species for colonization might differ and certain species may have difficulties to overcome dispersal barriers such as valleys or mountain ridges. The steeper relief and recent high rates of natural re-forestation at higher altitudes (Stöcklin et al., 2007) may also impose obstacles to dispersal.

The higher homogeneity in lowlands is most probably the result of increased invasion of generalists (see Table 2, Moradi et al., in prep). In addition, vascular plant specialists decreased within the 10–year observation period mostly at low altitudes (see Bergamini et al, 2009). However, as rare species might have a weak influence on distance-decay curves (Morlon et al., 2008) the clear increase in generalist and warm-temperature adapted species especially at low

altitudes (Moradi et al., in prep) might have had a higher influence on the decrease in similarity than the increased extinction rate of specialists from 1995/97→2005/06 (Bergamini et al., 2009).

Differences between management types

Decay of similarity over geographical distance was significant in mown fens in 2006, but not in grazed fens anymore. Hence, biotic homogenization may be reduced by changing management type from grazing to mowing. This might be desirable if cattle spread generalist and invasive species, whereas it could be negative if it reduces exchange of specialist species between sites. In general, grazing increases the dispersal distance of zoochorous species and the availability of germination safe sites (Harper 1977). The disturbance caused by cattle and therefore the increased availability of germination safe sites may have led to the higher floristic similarity between distant grazed than mown fens and low rate of distance decay after the 10-years period as especially common generalist plant species are often better adapted to disturbed sites than the specialized fen species and may benefit from an altered habitat quality at lower altitudes.

Differences between generalists and specialists

Compositional similarity between sites in particular in the first survey was higher for specialist than for generalist vascular plants. Grootjans et al (2006) in a review paper have shown that different hydrological landscape settings can maintain similar nutrient-poor and base-rich habitat conditions suitable for fen meadow species. Therefore, similar fen meadow communities can occur in different landscapes and regions. In this case, distance effects may be attenuated by the ecological stability of these habitats caused by special environmental conditions favouring adapted species. This would explain the high specialist' floristic similarity among our fens and the lower rate of decay over distance in compositional similarity of specialists than generalists.

In contrast to the compositional similarity regarding specialists, which can be considered an indication for the ecological integrity of these fen sites, the increasing compositional similarity regarding generalists maybe viewed as an undesirable effect of biotic homognization. During this homogenization specialized species can be replaced by ubiquitous species. Our results indicate such a replacement process because specialist species disappeared over the 10–year observation period, whereas generalists increased in species richness.

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Table 1 Location, altitudinal class, management type and geographical distance between pairs of the 36 studied fens. Altitudinal level (1: 800-1000m, 2: 1000-1200m, 3: 1200-1400m); Site No.: Code number according to the inventory of fens (BUWAL 1990).

| Municipality | Altitudinal class | Management | Site No. | Geographical distance (Km) | | |

Table 2 Mean alpha and gamma diversity in different subsets of vascular plant and bryophyte communities in the two surveys; mean ± standard error.

Data set	alpha diversity (1995-97)	alpha diversity (2005-2006)	gamma diversity (1995-97)	gamma diversity (2005-2006)
Vascular plants at 800-1000m	59.3 ± 2.71	62.0 ± 2.44	151	165
Vascular plants at 1000-1200m	61.4 ± 1.70	65.3 ± 1.88	154	160
Vascular plants at 1200-1400m	61.4 ± 3.49	62.3 ± 3.94	147	152
Bryophytes at 800-1000m	25.1 ± 1.05	22.8 ± 0.99	66	58
Bryophytes at 1000-1200m	26.0 ± 1.09	25.0 ± 0.98	69	77
Bryophytes at 1200-1400m	24.6 ± 1.28	25.0 ± 1.90	65	71
Vascular plants in mown fens	64.2 ± 2.16	67.6 ± 2.33	172	173
Vascular plants in grazed fens	56.7 ± 1.84	59.3± 1.92	148	164
Bryophytes in mown fens	24.4 ± 0.88	23.9 ± 0.88	67	66
Bryophytes in grazed fens	26.1 ± 0.93	24.6 ± 1.32	88	86
Specialist vascular plants	13.3 ± 0.55	12.4 ± 0.56	24	22
Generalist vascular plants	47.2 ± 1.40	50.8 ± 1.54	176	182
Specialist bryophytes	8.0 ± 0.29	7.2 ± 0.33	16	15
Generalist bryophytes	17.3 ± 0.65	17.0 ± 0.71	83	90

Table 3 Results of Mantel-tests for association between matrices of geographical distances and compositional similarities (Jaccard index) in different subsets of vascular plant and bryophyte communities at the two survey dates (1995/97 and 2005/06). (*): $P = 0.1$, *: $P = 0.05$, **: $P = 0.01$, ***: $P = 0.001$.

Data set	*P*-value (1995/97)	*P*-value (2005/06)
Vascular plants at 800–1000m	0.19	0.53
Vascular plants at 1000–1200m	0.001 ***	0.07 (*)
Vascular plants at 1200–1400m	0.002 ***	0.02 *
Bryophyte at 800–1000m	0.13	0.61
Bryophyte at 1000–1200m	0.03 *	0.03 *
Bryophyte at 1200–1400m	0.14	0.16
Vascular plants at mown fens	0.09 (*)	0.05 *
Vascular plants at grazed fens	0.00 ***	0.45
Bryophytes at mown fens	0.09 (*)	0.23
Bryophytes at grazed fens	0.07 (*)	0.19
Specialist vascular plants	0.45	0.51
Generalist vascular plants	0.07 (*)	0.24
Specialist bryophytes	0.83	0.39
Generalist bryophytes	0.03 *	0.62

Table 4 ANOVA table for main and interactive effects on vegetation similarity.

M: management, a: altitude (linear), A: altitudinal level (deviation from linearity), GeoDist: geographical distance between sites, Pair: Pairs of sites for which their vegetation similarity was related to geographical distance and other study design factors. It should be noticed that here the sites, which are located at the same altitudinal level and under the same management regimes were compared.

Source of variation	df	SS	% SS
M	1	0.3087	12.21
a	1	0.1758	6.95
A	1	0.0119	0.47
M:a	1	0.2272	8.99
M:A	1	0.2632	10.41
GeoDist	1	0.8407	33.25
M:GeoDist	1	0.1805	7.14
a:GeoDist	1	0.1187	4.70
A:GeoDist	1	0.2595	10.26
M:a:GeoDist	1	0.0338	1.34
M:A:GeoDist	1	0.1083	4.28
Pair	78	2.0245	

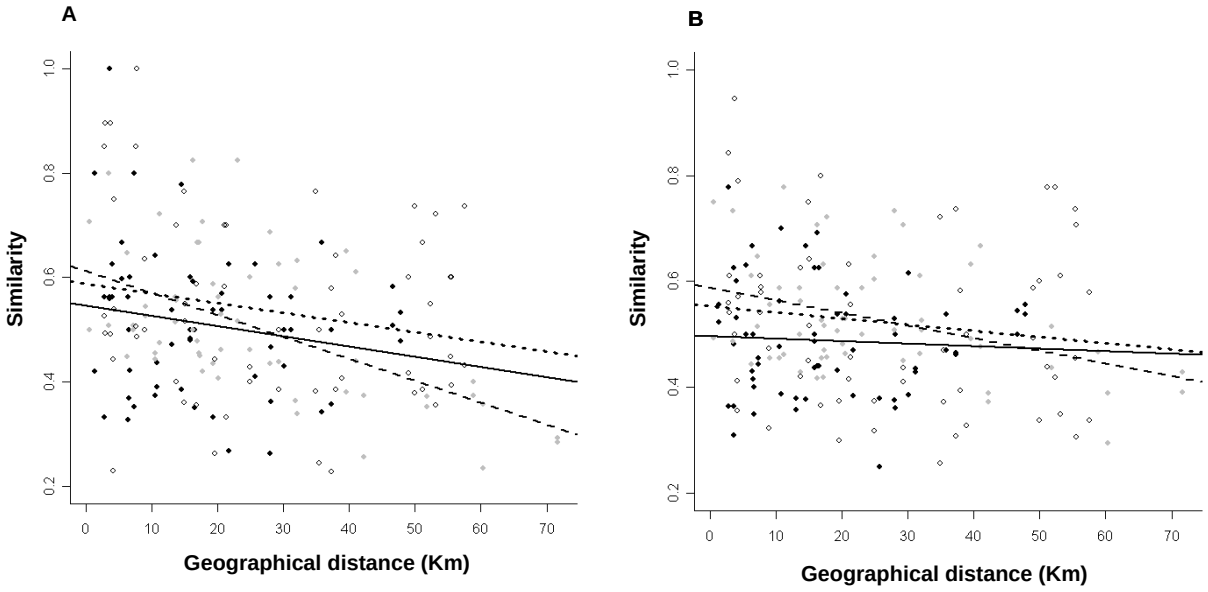

Figure 1 Decrease of compositional similarity of vascular plant communities with geographical distance at three altitudinal levels: — 800-1000m, ----: 1000-1200m, ….: 1200-1400m at a) the first survey (1995/97) and b) the second survey (2005/2006).

The distance-related decay of compositional similarity significantly decreased with geographical distance in 1995 at the middle (P= 0.001) and higher altitudinal levels (P= 0.002). In 2005/06 a significant distance-related decay of similarity was observed only at the highest altitudinal level (P=0.02)

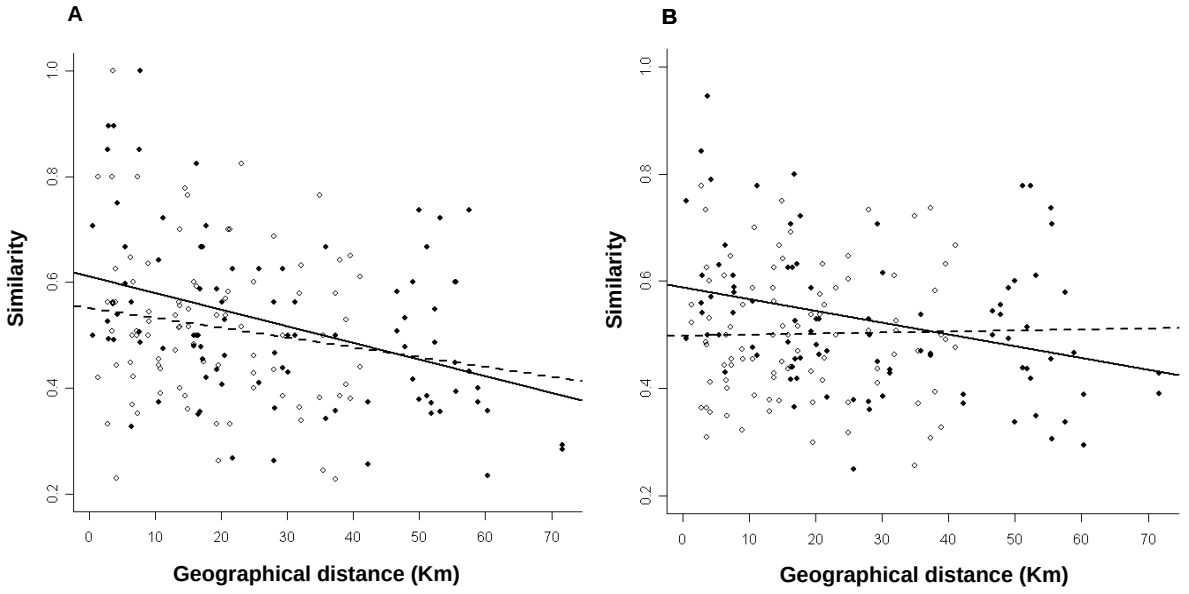

Figure 2 Decrease of compositional similarity of vascular plant communities with geographical distance in the two management types: —: Mowing, ----: grazing a) at the first survey (1995/97) and b) at the second survey (2005/06).

The distance-related decay of compositional similarity was highly significant among grazed fens in 1995 (P<.0.001), but not anymore in 2005/06 (P = 0.45). In mown fens, the similarity decay with distance was marginally significant in 1995 (P= 0.09) and significant in 2005/06 (P=0.05).

Appendix 1: Results of repeated measures analysis

ANOVA table: Response variable: Vegetation similarity.

M: Management, a: altitude, A: Altitudinal level, GeoDist: Geographical distance between sites, VasBry: Vascular plants or Bryophyte, GenSpe: Generalist or specialist species, Year: Year of survey, Pair: Pairs of the sites of which their vegetation similarity was related to geographical distance and other study design factors. It should be noticed that here the sites which are located in the same altitudinal class and under the same management regimes were compared.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
M	1	0.3087	0.3087	37.1571	4.46E-09	***
a	1	0.1758	0.1758	21.1582	6.93E-06	***
A	1	0.0119	0.0119	1.4326	0.232553	
M:a	1	0.2272	0.2272	27.355	3.76E-07	***
M:A	1	0.2632	0.2632	31.6834	5.17E-08	***
GeoDist	1	0.8407	0.8407	101.2116	< 2.2e-16	***
M:GeoDist	1	0.1805	0.1805	21.7254	5.29E-06	***
a:GeoDist	1	0.1187	0.1187	14.2873	0.000199	***
A:GeoDist	1	0.2595	0.2595	31.2364	6.33E-08	***
M:a:GeoDist	1	0.0338	0.0338	4.0639	0.044953	*
M:A:GeoDist	1	0.1083	0.1083	13.0363	0.000374	***
Pair	78	2.0245	0.026	3.1246	1.41E-11	***
VasBry	1	0.0536	0.0536	6.4583	0.01169	*
GenSpe	1	6.0108	6.0108	723.6175	< 2.2e-16	***
VasBry:GenSpe	1	0.0794	0.0794	9.5545	0.002236	**
M:VasBry	1	0.1544	0.1544	18.5879	2.39E-05	***
a:VasBry	1	0.011	0.011	1.3295	0.250068	
A:VasBry	1	0.0213	0.0213	2.5632	0.110723	
M:a:VasBry	1	0.084	0.084	10.1152	0.001669	**
M:A:VasBry	1	0.0015	0.0015	0.1865	0.666269	
GeoDist:VasBry	1	0.0064	0.0064	0.7719	0.380525	
M:GeoDist:VasBry	1	0.0003	0.0003	0.0315	0.859322	
a:GeoDist:VasBry	1	0.1597	0.1597	19.2268	1.76E-05	***
A:GeoDist:VasBry	1	0.028	0.028	3.3655	0.067844	.
M:a:GeoDist:VasBry	1	0.0597	0.0597	7.1926	0.007842	**
M:A:GeoDist:VasBry	1	0.0044	0.0044	0.5243	0.469742	
M:GenSpe	1	0.0068	0.0068	0.8223	0.365428	
a:GenSpe	1	0.6355	0.6355	76.4997	4.39E-16	***
A:GenSpe	1	5.58E-06	5.58E-06	0.0007	0.97934	
M:a:GenSpe	1	0.0172	0.0172	2.0763	0.150939	
M:A:GenSpe	1	0.0608	0.0608	7.3144	0.007343	**
GeoDist:GenSpe	1	0.049	0.049	5.9	0.015895	*
M:GeoDist:GenSpe	1	0.1177	0.1177	14.1728	0.000211	***
a:GeoDist:GenSpe	1	0.1354	0.1354	16.2947	7.35E-05	***
A:GeoDist:GenSpe	1	0.0419	0.0419	5.0473	0.025597	*
M:a:GeoDist:GenSpe	1	0.0233	0.0233	2.8044	0.095343	.
M:A:GeoDist:GenSpe	1	0.18	0.18	21.6643	5.44E-06	***
M:VasBry:GenSpe	1	0.0332	0.0332	3.9982	0.046706	*
a:VasBry:GenSpe	1	0.0598	0.0598	7.2051	0.007789	**

A:VasBry:GenSpe	1	0.0988	0.0988	11.8989	0.000666	***
M:a:VasBry:GenSpe	1	0.0274	0.0274	3.295	0.070771	.
M:A:VasBry:GenSpe	1	0.1314	0.1314	15.8133	9.32E-05	***
GeoDist:VasBry:GenSpe	1	0.0049	0.0049	0.5854	0.444956	
M:GeoDist:VasBry:GenSpe	1	0.0002	0.0002	0.026	0.871998	
a:GeoDist:VasBry:GenSpe	1	0.0033	0.0033	0.3913	0.532233	
A:GeoDist:VasBry:GenSpe	1	0.0001	0.0001	0.0076	0.930409	
M:a:GeoDist:VasBry:GenSpe	1	0.072	0.072	8.6674	0.003566	**
M:A:GeoDist:VasBry:GenSpe	1	0.027	0.027	3.2454	0.072913	.
Pair:VasBry:GenSpe	234	2.7539	0.0118	1.4168	0.003964	**
Year	1	0.0187	0.0187	2.2509	0.134886	
M:Year	1	0.0596	0.0596	7.1752	0.007916	**
a:Year	1	0.0003	0.0003	0.0377	0.846258	
A:Year	1	0.0388	0.0388	4.6731	0.031653	*
M:a:Year	1	0.0001	0.0001	0.0148	0.903241	
M:A:Year	1	0.0005	0.0005	0.0601	0.806595	
GeoDist:Year	1	0.0203	0.0203	2.4383	0.119757	
M:GeoDist:Year	1	0.0002	0.0002	0.0183	0.892488	
a:GeoDist:Year	1	0.0058	0.0058	0.6942	0.405596	
A:GeoDist:Year	1	0.0012	0.0012	0.1488	0.700074	
M:a:GeoDist:Year	1	0.028	0.028	3.3739	0.067506	.
M:A:GeoDist:Year	1	0.0035	0.0035	0.4208	0.517179	
Pair:Year	78	0.5985	0.0077	0.9237	0.653389	
VasBry:Year	1	0.0035	0.0035	0.4218	0.516701	
GenSpe:Year	1	0.1062	0.1062	12.7794	0.000426	***
VasBry:GenSpe:Year	1	0.0031	0.0031	0.372	0.542507	
M:VasBry:Year	1	0.0098	0.0098	1.1802	0.278429	
a:VasBry:Year	1	0.0031	0.0031	0.3718	0.542605	
A:VasBry:Year	1	0.0043	0.0043	0.5164	0.473118	
M:a:VasBry:Year	1	0.0178	0.0178	2.1386	0.144976	
M:A:VasBry:Year	1	0.0026	0.0026	0.3131	0.576331	
GeoDist:VasBry:Year	1	0.0081	0.0081	0.9775	0.323826	
M:GeoDist:VasBry:Year	1	0.0056	0.0056	0.6765	0.411642	
a:GeoDist:VasBry:Year	1	0.0024	0.0024	0.2831	0.595163	
A:GeoDist:VasBry:Year	1	0.0083	0.0083	1.0025	0.317747	
M:a:GeoDist:VasBry:Year	1	0.0035	0.0035	0.4171	0.519021	
M:A:GeoDist:VasBry:Year	1	0.0056	0.0056	0.673	0.412845	
M:GenSpe:Year	1	0.0424	0.0424	5.1023	0.024815	*
a:GenSpe:Year	1	0.0004	0.0004	0.0526	0.81872	
A:GenSpe:Year	1	0.0511	0.0511	6.1563	0.013798	*
M:a:GenSpe:Year	1	0.0012	0.0012	0.1487	0.700141	
M:A:GenSpe:Year	1	0.0044	0.0044	0.5323	0.466373	
GeoDist:GenSpe:Year	1	0.0014	0.0014	0.1691	0.681248	
M:GeoDist:GenSpe:Year	1	0.0068	0.0068	0.8229	0.365268	
a:GeoDist:GenSpe:Year	1	0.0001	0.0001	0.0138	0.906607	
A:GeoDist:GenSpe:Year	1	0.0066	0.0066	0.7989	0.372338	
M:a:GeoDist:GenSpe:Year	1	0.0065	0.0065	0.7823	0.377352	
M:A:GeoDist:GenSpe:Year	1	0.0086	0.0086	1.038	0.30934	
M:VasBry:GenSpe:Year	1	0.0753	0.0753	9.062	0.002896	**
a:VasBry:GenSpe:Year	1	0.0076	0.0076	0.9185	0.338849	
A:VasBry:GenSpe:Year	1	0.017	0.017	2.0506	0.153483	
M:a:VasBry:GenSpe:Year	1	0.007	0.007	0.846	0.358646	
M:A:VasBry:GenSpe:Year	1	0.0194	0.0194	2.3409	0.127363	
GeoDist:VasBry:GenSpe:Year	1	0.0275	0.0275	3.3156	0.069901	.
M:GeoDist:VasBry:GenSpe:Year	1	0.004	0.004	0.4857	0.486547	
a:GeoDist:VasBry:GenSpe:Year	1	0.0178	0.0178	2.137	0.145124	
A:GeoDist:VasBry:GenSpe:Year	1	0.0034	0.0034	0.4135	0.520807	
M:a:GeoDist:VasBry:GenSpe:Year	1	0.001	0.001	0.1229	0.726173	
M:A:GeoDist:VasBry:GenSpe:Year	1	0.0041	0.0041	0.4917	0.483862	
Residuals	234	1.9437	0.0083			

Chapter 3

Effects of landscape structures on recent change in species diversity of protected wetland ecosystems

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Abstract

This study investigates how landscape and fen characteristics (patch size, distance between fens, number of neighbouring fens, management type), surrounding habitat types, and potential landscape permeability may influence vascular plant and bryophyte species diversity. In addition, we assessed the temporal change of the spatial effects in these protected wetlands within the last 10 years. Even though calcareous fen meadows are legally protected since 1987, the abundance of this special vegetation type in Switzerland has further decreased by 10% within the last 10 years due to conversion of these fens into other wetland vegetation types. We studied an archipelago of 36 species-rich calcareous fen meadows identifiable by their typical *Carex davalliana* vegetation type in the Swiss pre-Alps (800–1400m a.s.l.) for two time steps (1995/97, 2005/06). Our randomly chosen fens were all traditionally managed, but differed in the type of management (mown vs. grazed). Inter-patch distance had the most consistent and strongest influence on plant diversity in our fen meadows whereas area-based metrics were less important, at least in the first census. The effects of spatial isolation of a habitat patch were stronger for specialist than generalist species. Total vascular plant species density, and specialist density as well as the Shannon and Simpson index were higher in fens with shorter distance to a neighbouring patch. Specialist vascular plant species density was positively affected by the number of habitat neighbours and negatively affected by the presence of potential dispersal barriers, i.e. by vegetation types other than open grassland around the focal patches, whereas generalist vascular plants were not influenced by dispersal obstacles. For bryophytes that are good dispersers, we found a less clear relationship between landscape metrics and species density except for obstacles to dispersal, which negatively affected bryophyte species density at the second census in 2005/06. In general, effects of landscape structure on plant biodiversity, i.e. especially on specialist species density, were more pronounced at the time of the second census in 2005/06, suggesting lag effects of habitat fragmentation that should therefore be considered in conservation planning.

Keywords: Spatial patterns, landscape permeability, vascular plants, bryophytes, specialist plants, generalist plants, species diversity, spatial isolation, habitat fragmentation

INTRODUCTION

Background

Ecological systems are heterogeneous in space and time. In landscape ecology, spatial heterogeneity is usually referred to as landscape structure or landscape patterns. Changes in landscape patterns through time are generally referred to as landscape dynamics (Turner et al., 2001). Among structural properties, the spatial arrangement of landscape elements and the interaction distances among biotic elements play a major role in driving landscape processes and in determining community compositions (Forman 1995; With 1997; Klopatek & Gardner 1999, Mesquita et al., 1999; Bolliger et al., 2003; Green & Sadedin 2005; Hersperger 2006). Interaction distances among biotic elements within a landscape not only influence environmental patterns (Green & Sadedin 2005; Taylor et al., 1993; With et al., 1997), but do also significantly influence the ecosystems' tolerance and resilience to disturbance. Consequently, the distribution of habitats and dispersal abilities of organisms define the capacity for the ecosystem to respond to perturbation and the connectedness of landscape elements (Gardner et al., 1987; Kindlmann et al., 2005; O'Neill et al., 1988; With & Crist 1995).

Environmental perturbations are especially frequent and intense in modern, human-dominated landscapes. For example, anthropogenically induced changes of landscape elements often create islands of natural habitat embedded in an unsuitable matrix (Groom & Schumaker 1993; Hanski 1999; Cook et al., 2002). Such alterations of landscape structures lead to fragmentation, i.e. to a decrease in habitat patch size and often to a concomitant increase of inter-patch habitat distances (Goodwin & Fahrig 2002), which both decrease interaction distances in landscapes. Therefore, habitat fragmentation may hamper dispersal, which is critical for the long-term population viability of many species (e.g. Debinski & Holt 2000; Laurance et al., 2002; Lienert 2004; Joshi et al., 2006). In fragmented landscapes, species compositions are altered because not all species respond equally to changed landscape

patterns (Tscharntke et al., 2002). Some species are more vulnerable than others to reduced area, respectively to reduced population size, and to increased isolation and edge effects that accompany the fragmentation process (e.g., Lienert 2004). Habitat specialists are often more affected by habitat loss and isolation than generalists that also may survive in the matrix habitat (Fischer & Stöcklin 1997; Jonsen & Fahrig 1997; Warren et al., 2001; Cook et al., 2002; Joshi et al., 2006). In addition, species also differ in the rapidity of response; and often severe effects of habitat fragmentation on biodiversity may only be visible in the long-term (e.g. Robinson et al., 1992; Kareiva & Wennergren 1995; Debinski & Holt 2000).

To date, many researchers have focused on the effects of patch size on species richness (e.g. MacArthur & Wilson 1967; Gutzwiller & Anderson 1992; Holt 1993) and on the effects of habitat isolation, inter patch-distance and landscape connectivity on the behaviour of a single or few species (Taylor et al., 1993; Fahrig & Merriam 1994; Hanski 1999). However, there is a lack of studies on the importance of landscape patterns on species migration and extinction risks in fragmented landscapes under environmental change (Jump & Penuelas 2005; Hersperger 2006).

What is the relationship between landscape spatial characteristics and fen species diversity?

In this study, we assessed 1) the effects of landscape spatial patterns and potential landscape permeability on vascular plant and bryophyte diversity in species-rich fen ecosystems, and 2) investigated the possible temporal change of the spatial effects in these protected wetlands over a 10-year observational period.

Both patch size and inter-patch distances are important determinants of immigration and extinction processes (MacArthur & Wilson 1967; Debinski & Holt 2000) that influence the diversity patterns in the landscape. We studied an archipelago of 36 species-rich calcareous fen meadows identifiable by their typical *Carex davalliana* vegetation type in the foothills of

the Swiss Alps (800–1400m a.s.l.) in 1995-97 and 2005-06 (see below). The randomly chosen fens were all traditionally managed, but differed in the type of management (mown once in autumn vs. extensively grazed by cows) and altitudinal class (800–1000, 1000–1200, 1200–1400 m a.s.l.) according to a balanced factorial design. For each of the six possible factorial combination: management type × altitudinal class, there were six replicates (Bergamini et al., 2001b; Bergamini et al., 2009). Furthermore, the selection was done in a way to avoid a confounding of site area with the classification factors listed above (Bergamini et al., 2001b). These calcareous fen meadows harbour a specialized and species-rich type of plant community adapted to the nutrient-poor and moist site conditions (Ellenberg 1996). In Switzerland, calcareous fen meadows are naturally patchy. However, within the last century, the wetland area in Switzerland has decreased by more than 90 % (Klaus 2007). Even though calcareous fen meadows are legally protected since 1987 (Grünig 1994), the abundance of this special vegetation type has decreased by further 10% within the last ten years due to conversion of the typical *Carex davalliana* vegetation type into less specialised wetland vegetation types (Klaus 2007).

Specifically, we tested how landscape and fen characteristics (patch size, distance between fens, number of neighbouring fens, management), surrounding habitat types and potential landscape permeability may influence plant and bryophyte species diversity. The following questions were investigated:

1.1 Does plant-species diversity increase with increasing fen size? Premise for this species-area relationship (Arrhenius 1921; Gleason 1922; May 1975) is that larger patches of habitat allow bigger population sizes and contain more resources and larger heterogeneity (Kohn & Walsh 1994), therefore allowing more niches and more species to coexist than small habitat areas

1.2 Does spatial distance between fens influence species density? We hypothesize that fens located closely to each other exhibit a higher species density than fens located at greater distances since the level of dispersal among local habitat patches in a metacommunity determines species richness and evenness patterns of a patch (Hubbel 2001; Hoyle 2005; Holyoak et al., 2005).

1.3 What is the relative importance of different local environmental factors on fen species density? Are altitude, different management types (mowing vs. grazing), climatic variables and interactions of the factors listed above driving fen species diversity? We hypothesize that colonization dynamics are mainly driven by factors, which determine the arrival of a species at a patch, acting at the landscape scale, whereas establishment and persistence are mostly determined by local environmental factors that operate within the patch (Wright et al., 2003).

In addition, we investigated the dynamics of the relationship between landscape properties and colonization and extinction events during the past 10 years. This assessments on the change in species occupancies relied on a repetition of fen-vegetation surveys in 2005/06, which were initially conducted in 1995 for vascular plants and 1997 for bryophytes (Wettstein & Schmid 1999; Bergamini et al., 2001b; Pauli et al., 2002; Peintinger et al., 2003). We specifically were interested in the following dynamical patterns:

2.1 Are effects of patch size and isolation on plant species diversity more pronounced in the second census of 2005/06 than in 1995/97? We hypothesize that effects on smaller fens with high isolation should be more pronounced in the second census of 2005/06 than back in 1995/97 since fragmentation and isolation of patches can cause a loss of species that is nonlinear and delayed for a long time due to a time-lag effect of long-lived species that survive in fragments but cannot reproduce anymore (Tilman et al. 1994; Kareiva & Wennergren 1995; Debinski et al., 2000; Laurance et al., 2002; Joshi et al., 2006).

2.2. Do specialist and generalist species differ in their response patterns over time? We hypothesize that fen specialists are becoming more responsive to land-use change with time, since they have a lower probability of crossing boundaries (Wiens et al., 1985; Haddad 1999), and are likely to experience a higher mortality while dispersing through the matrix (Tischendorf et al., 2003).

METHODS

Field sites

In total, 36 montane calcareous fen meadows of the phytosociological order *Caricion davallianae* (Ellenberg 1996) were studied in the Swiss pre-Alps. Calcareous fen meadows are of high conservation concern due to their high biodiversity (Wettstein & Schmid 1999; Bergamini et al., 2001; Peintinger et al., 2003). Our study region covered an area of approximately 3500 km^2 in East and North-eastern Switzerland (see Fakheran et al. in prep. for a complete list of the study sites). The sites were randomly selected from over 600 fens listed in a national inventory (BUWAL 1990; Bergamini et al., 2009). Annual precipitation in our study region was high (1500–2800 mm) and bedrock mainly consisted of various calcareous sediments of Tertiary and Mesozoican age (Spicher 1972). Our selection was stratified into two traditional management types (mown once in early autumn vs. extensively grazed by cows) and three altitudinal levels (800–1000, 1000–1200, 1200–1400 m a.s.l.). At each altitudinal level, we had 12 sites with a difference in elevation of up to 200 m among sites. The soil of our fen sites was nutrient-poor and base-rich (Bergamini et al. 2001b; Pauli et al., 2002). In previous studies, we showed that the management of the protected wetlands affected the plant-species composition of the studied fens with some fen taxa benefitting from grazing and others from mowing (vascular plants: Peintinger 1999; Bergamini et al., 2009; bryophytes: Bergamini et al. 2001b; butterflies and grasshoppers: Wettstein & Schmid 1999). On the landscape level, a mixture of both management types was therefore recommended for

the long-term protection of fen taxa (Wettstein & Schmid 1999; Peintinger 1999; Bergamini et al., 2001b).

Vegetation monitoring

In 1995, the presence/absence of vascular plants and in summer 1997, the presence/absence of bryophytes was recorded at all 36 sites. Each site was sampled using five randomly selected plots of 2 ×1 m, giving a total of 180 plots distributed over the 36 fens (see Bergamini et al., 2009 for a more detailed description of the monitoring process and the nomenclature used). The second survey took place in July and August 2005 (24 randomly selected fens out of the totally 36 fens) and July 2006 (12 fens). Shape and size of plots were identical to the first survey. The management changed in one of the mid-altitudinal sites from grazing to mowing within the study period. However, as the site was for a longer time period grazed than mown, we regarded this site as grazed in our analyses.

Specialists and non-specialists

Twenty-five species which are listed in characteristic of the *Caricetalia davallianae* vegetation type in BUWAL (1990) were designated as habitat specialists. All other species were defined as generalists.

Species diversity

Species presence/ absence data were used to calculate the cumulative species density of specialist, generalist and all species at site level, i.e., at the level of the 5 plots per site (10 m^2). Evenness and dominance patterns were analysed using Shannon and Simpson diversity indices calculated for each site in both census periods. Shannon's diversity index (H') was calculated as $H' = \Sigma pi \ ln(pi)$, where pi denotes the proportional abundance of the i^{th} species (Magurran 2004). The Shannon index takes the number of species and the evenness of the

species into account and increases either by having additional unique species, or by having greater species evenness. It can be shown that for any given number of species, there is a maximum possible (H'_{max}), H'_{max} = ln (species richness), which occurs when all species are present in equal numbers. In a sample with only one species, H' becomes 0.

The Simpson Index (D) was estimated as $D=\Sigma pi^2$. The Simpson Index gives the probability that two individuals drawn at random from a community belong to the same species (Simpson 1949).

Spatial structural fen properties and landscape context

Metrics to characterise the spatial structure of fens were quantified using **ArcGIS 9.00** (ESRI 2004) and include (see Table 1):

- Area (area of the focal fen patch in m^2)
- *ND* (Nearest Distance to the next fen in m)
- *Buffer* (Number of neighbouring fens around the focal fen within a 1000 or 2000 m buffer zone)
- *NN-Area* (area of the nearest neighbour to the focal fen in m^2)

Distances between patches were measured edge-to-edge instead of using central site co-ordinates to take into account the closer distance between adjacent long narrow strips of rectangular habitats arranged end-to-end compared to the center-to-center distance (Bustin & Thomas 1999). We measured the nearest distance to the next fen considering all habitat patches in the landscape rather than only our 36 intensively studied fragments.

The landscape context of the fens was characterised by thematic land-use and land-cover data available for the whole of Switzerland (BFS 1992/97). The original data were derived from aerial photographs at a 100 m resolution, categorized into 74 land-use and land-cover classes. For the purpose of this study, the 74 land-use and land-cover classes were grouped into four classes (forest, streams, settlement, open land (= grassland and meadows)).

These classes were used to characterise the landscape within a 1000 or 2000 m radius of the investigated fens. Within these radii, the absence/presence of the four land-use/land-cover classes forest, streams, settlement, open land) was assessed and then further merged into a contrast of open grassland only vs. the neighbourhood containing solely or partly forests, streams or settlements. This contrast estimates the potential landscape permeability or therefore the obstacles to dispersal (Table 1):

- *Potential landscape permeability:* the presence of forests, streams or settlements within a radius of 100 or 200 m around a fen patch were considered dispersal barriers, whereas open land was assumed to be no obstacle to dispersal.

Altitude was defined as the altitude of the central plot at each site in m a.s.l. (see Bergamini et al., 2009, Moradi et al., in prep).

Climatic variables

Climatic variables were available as continuous surface maps, based on spatially interpolated data from standardized meteorological recordings (1960–2000) and a digital elevation model of Switzerland (DEM, 25 m). Degree-days (5 degree C threshold), the mean monthly precipitation sum, and the average monthly global potential shortwave radiation (kJ / day) were used (Table 1). For details on the calculation of climate variables, see Zimmermann & Kienast (1999). To account for the local climatic conditions and to have more meaningful climate-variable treatment than just the overlay of the fens with the climate variables, we considered neighbourhoods of 250 m around each fen and subsequently calculated mean, standard deviation, maximum, and minimum for degree days and the precipitation sum.

Statistical Analysis

To relate fen-species diversity to spatial structural properties, to a landscape context and to environmental variables (Table 1), the statistical software R was used (Version R 2.4.1 for

Windows; R development Core team 2007). The selected models were of the GLM (general linear model). Stepwise regressions were carried out to identify the best model fit comparing the R^2 of the different models to explain the variation in species diversity. The nearest distance to the next fen was log transformed.

To analyse changes in species diversity between 1995/97 and 2005/06, we used the following dependent variable (all measured at site level) for vascular plants: 1) total species density, using the cumulative number of species of 5 plots of 2 × 1 m (= species number per 10 m^2), 2) density of fen specialists, 3) density of generalists, 4) Shannon diversity index (based on cumulative species presence/absence data per 10 m^2), 5) Simpson diversity index (based on cumulative species presence/absence data per 10 m^2).

For bryophytes we used the following two dependent variable: 6) total species density, 7) density of specialist bryophyte species.

Plant diversity data in our study area were also analyzed with repeated-measures analyses of variance to test the influences of the landscape metrics and of the following factors: time, management, altitudinal class, climatic variables, and the interactions of these factors. The effects of landscape metrics, management, altitude, climatic variables and their interactions were tested against the random effects of sites (see also Bergamini et al., 2009; Moradi et al., in prep).

RESULTS

Do spatial landscape properties influence diversity patterns?

Does plant-species diversity increase with increasing fen size?

In total, 229 vascular plant and 120 bryophyte species were identified on all 36 fen sites during the two census periods. Fen size varied from 0.87 to 67.27 ha (Table 2). Total species density of vascular plants was positively affected by increasing fen size in both censuses (Table 3, Table 4). In contrast, fen specialist species were overall not influenced by patch size

(Table 4). However, separate analyses for each census date revealed that fen size did influence specialist species density in 2005/06, but not ten years earlier in 1995 (Fig. 1, Table 3): In 1995, species density of habitat specialists was equally high in small and in large habitat fragments, whereas 10 years later the density of specialist species mainly dropped in the smallest fragments (Fig. 1). The Shannon (but not Simpson) diversity of vascular plant species per 10 m2 also increased significantly with fen size (Table 4). However, both the Shannon and Simpson diversity index were high even in small fragments (Table 3). As shown in Table 2, the maximum Shannon index in the second census was 4.14. Usually, the value of the Shannon diversity index obtained from empirical data falls between 1.5 and rarely surpasses 4.0 (Margalef 1972 in Magurran 2004) and does not seem to exceed 5.0 in biological communities (Washington 1984 in Krebs 1989). In contrast to vascular plants, bryophyte species density was neither affected by patch area in the first nor in second census (Table 3, 4).

Does distance between fens influence species density?

The nearest distance to the next fen varied from 134.6 to 2657 m (Table 2). The nearest distance to the next habitat patch strongly influenced vascular plant species density and diversity (Table 4). Total vascular plant species density, and specialist density as well as the Shannon and Simpson index were higher in fens with shorter distance to a neighbouring patch (Figs 2, 3; Table 4). Non-specialist density, however, was not influenced by the nearest distance to the next fen. The Simpson index especially increased at the second census at the sites with the nearest distance to the next habitat patch (log ND × Census: Table 4; Fig. 2), whereas the slope of the non-specialist species density with nearest distance remained the same after ten years albeit on a overall higher level of species density (Fig. 3; Table 2). In contrast to vascular plants, the density of total and also of specialist bryophyte species was not affected by the nearest distance to the next fen habitat (Table 3, 4).

Area of the nearest neighbour fen (NN-Area)

The area of the nearest neighbour ranged between 0.58 and 12.65 ha (Table 2). In general, the area of the nearest neighbour affected vascular plant, but not bryophyte species (Table 4). An increase in neighbouring patch size had especially a positive effect on the Shannon index at the second census (Table 4, Neighbour area × census). But also total vascular plant and the density of generalist species increased with area of the nearest neighbour in the second census (Table 3, Fig. 7). Species density of bryophytes however was not affected by size of the neighbouring fen (Tables 3, 4).

How does the landscape context influence diversity patterns over time?

Number of neighbouring fens

Within a 1 km radius, the number of neighbouring fens varied from 0 to 29, and within a 2 km radius from 0 to 55 adjoining habitat areas (Table 2). The number of habitat neighbours had a strong effect on specialist species density both within a 1-km and 2-km buffer zone at both census dates (Table 4, Fig. 4) but did not influence generalist and total species density of vascular plants and bryophytes (Table 4). The density of vascular plant specialist species decreased especially in the second census at sites with no or few neighbouring habitat patches around (Fig. 4, Table 3).

Potential landscape permeability

The presence of potential dispersal barriers ("obstacles") within a radius of 100 m around the focal patches had a significantly negative effect on the density of specialist vascular plant species in both censuses (Table 4) with a 16.5% decline on average in specialist species density if other land-use types than grasslands were around the focal patch (Fig. 5). Total species density in the first census was also negatively affected by presence of potential dispersal barriers (58.77 ± 1.85 species/10 m^2 vs. 65.00 ± 2.56, Table 3), but not in the second

census (62.35 ± 1.92 vs. 65.56 ± 3.58, P > 0.2; Table 3) where, overall, a higher species density especially of habitat generalist species was observed (Moradi et al., in prep). In contrast to fen specialists, the density of non-specialist species was neither influenced by obstacles in the first nor in the second census (Table 4). Similarly, dispersal barriers did not significantly influence evenness and dominance patterns of vascular plant species (Shannon and Simpson diversity indices) at the studied sites (Table 4).

The total density of bryophyte species was not affected by obstacles in the first, but in the second census (Table 3; marginally significant effect of obstacles × census in Table 4) where bryophyte species density per site was lower with obstacles around them (Fig. 6).

Interactive effects of spatial landscape properties in 1995/97 vs. 2005/06

The interaction of log nearest distance × number of neighbouring fens within a 1-km buffer zone especially affected habitat specialists (Table 4): specialist species density decreased strongly in isolated fragments with a long distance to the next patch and few immediate neighbours around. Also bryophyte species density was affected by the interactive effect of log nearest distance × number of neighbouring fens within a 1-km buffer zone and was decreased if there were few immediate neighbours around especially in the first census (Table 4, nearest distance × number of neighbouring fens × census). A temporal change of the combined effect of nearest distance and neighbour number within a 2 km buffer zone was also observed for total vascular plant species density as well as for the Simpson and Shannon effect (Table 4).

Altitude and management

Management had a significant influence on species density of all groups analysed except specialist bryophytes that were not and specialist vascular plants, whose density was only marginally significantly influenced by management (Table 4). Total species density of

vascular plants was higher in mown than in grazed fens at both census dates (65.89 ± 1.59 species/10 m^2 vs. 57.81 ± 1.3 management × census; see also Bergamini et al. 2009) and species density increased with altitude in mown, but not in grazed fens (Table 4; see also Moradi et al., in prep). In contrast, specialist species density increased with altitude independently of management regime (Table 3, Moradi et al., in prep). Species density of bryophytes, however, did not depend on altitude (Table 4) in mown but in grazed fens (Table 4: management × altitude, see also Bergamini et al., 2009). There was no change in the influence of management for any group between census dates (repeated measures analyses all $P > 0.1$). The effect of altitudinal class on the Simpson index, however, changed among census dates (Table 4).

Climatic variables

Overall, the climatic variables tested did not have a significant influence on species density of any group. Only the mean precipitation sum in July had a positive significant effect on density of specialist vascular plants (Table 4). Monthly average shortwave radiation had an effect on the Simpson index (Table 4). The influence of the maximum annual degree days on species diversity, both on the Shannon and Simpson index, changed over time (Table 4): in the second census, in contrast to the first census, both Simpson and Shannon diversity indices increased with an increase in the maximum annual degree days.

DISCUSSION

Spatial landscape properties

In general, distance-based isolation metrics were more important to predict plant diversity patterns in the calcareous fen meadows than were area-based metrics. In 1995, biodiversity was high even in small fragments, mainly species density of vascular fen specialist was equally high in small as in large fens (Fig. 1) suggesting a high conservation value even for

small habitat patches (Peintinger et al., 2003). Given the longevity of typical fen species that are predominantly clonal (Moradi et al., in prep) it is not surprising that rapid recovery of species diversity has been observed even 35 years since abandonment of traditional management (Billeter et al., 2007), thus a reduction in habitat area due to fragmentation might primarily lead to a decreased reproduction and species abundance but not to immediate species extinctions and therefore reduced species densities (Hooftman & Diemer 2002). The drop in specialist species density in small habitat fragments after a decade in 2005/06, which was detected in the analyses, supports the assumption of a delayed response of fen specialists to fragmentation. A time-lag effect of habitat fragmentation has also been observed in dry calcalreous grasslands (Joshi et al 2006) and lowland tropical rain forests (Laurance et al., 2002). Genetics studies of once abundant fen specialists suggest that a reduction in patch size combined with an increased isolation leads to loss of genetic diversity and fitness in these long-lived species (e.g. Schmidt & Jensen 2000; Billeter et al., 2002; Lienert et al., 2002; Hooftman et al., 2004; Galeuchet et al., 2005). For example, small population size alone did not significantly reduce genetic variability in *Swertia perennis*, a fen specialist, in habitat patches, which were close to large islands, and therefore presumably experienced sufficient gene flow (Lienert et al., 2002). However, if small population size was combined with geographic isolation, genetic variability declined significantly (Lienert et al., 2002). Accordingly, in our study, the number of neighbouring fens as well as distance-based isolation metrics had a significant influence on fen specialist density especially in the second census. For fen specialists, which are restricted to fen fragments and unable to use the matrix habitat around, the number of neighbouring fen patches both within a 1 km and 2 km buffer zone, the distance to the next fen, as well as the presence of obstacles to dispersal, i.e. the presence of adjoining land-use elements other than grassland vegetation, was crucial to maintain the original species densities. Some other studies also demonstrated that species richness was closely linked with landscape characteristics at a regional scale (Dunning et al.,

1992; Dale et al. 2000; Wagner et al. 2000) and that inter-patch distance had a strong effect on connectivity between populations (With & King 1999; Tischendorf & Fahrig 2000a; Goodwin & Fahrig 2002). Therefore, it is justified that conservation strategies now frequently also consider the surrounding landscape context that influences the habitat patches (e.g. Shumaker 1996).

Distance-based isolation metrics did also influence generalist and total species density especially in the second census, which increased log-linearly with shorter distance to the next fen. This coincides with an overall increase in species density observed after a decade, despite a drop in species number of fen specialists, both at the plot level (Bergamini et al., 2009) and at the site level (Moradi et al., in prep). This increased species richness was mainly caused by an increased colonisation of warm-temperature adapted plants and ubiquitous as well as shade tolerant species (Moradi et al., in prep) leading to an overall homogenization of fen community composition (Fakehran et al., in prep).

Vascular plants vs. bryophytes

In contrast to vascular plants, for bryophytes, we found a less clear relationship between distance-based isolation metrics and species density except for dispersal obstacles, which negatively affected bryophyte species richness after ten years in 2005/06. Total bryophyte species richness was mainly explained by management, altitude and precipitation. These results could be explained in part by the fact that bryophytes are good dispersers (Miles & Longton 1992) and also by the smaller size of bryophytes which may allow them to persist in micro sites in the matrix habitats, which are not available for vascular plants (Bergamini et al., 2009).

Specialist vs. generalist species

Our study indicates that the effects of spatial isolation of a habitat patch are stronger for specialist than generalist species. Especially at the second census, fen specialists were more affected by patch size, nearest distance to the next patch, number of habitat neighbours and area of the nearest habitat patch than generalists. In addition, specialist species showed also more pronounced negative responses to surrounding land use (dispersal barriers) than did generalist species.

CONCLUSION

We conclude that conservation strategies should consider not only the habitats, but also the surrounding landscape context that influences species richness in habitat patches. Spatial arrangement of the habitat patches as well as the interactive effects of landscape metrics are important concerns in conservation of fens in Switzerland.

This study also revealed that conservation planning should take into account the lag effects of habitat fragmentation on plant species diversity, because some fragmentation effects will only be apparent and pronounced after decades.

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Table 1 Spatial structural and environmental variables used to perform the presented study.

	Abbreviation	Description
Landscape metrics	Area	Fen area (m^2).
	NN-Area	Area (m^2) of the nearest neighbouring fen.
	ND	Distance (m) to the nearest fen (edge-to-edge distance).
	Buffer-1km	Number of neighbouring fens in a 1000 m radius around the focal fen.
	Buffer-2km	Number of neighbouring fens in a 2000 m radius around the focal fen.
Landscape context	Potential landscape permeability	Thematic landscape information within 100 m neighbourhood of the edge of a fen favouring/hindering seed dispersal.
	Altitude	m above see level (from digital elevation model, 25 m).
Climate variables	Degree days	5 degree C threshold (count) (for details see Zimmermann & Kienast 1999).
	Monthly mean precipitation	mm (for details see Zimmermann & Kienast 1999).
	Srad	Monthly global potential shortwave radiation (kJ / day).
Management	Man	Grazing: extensive grazing by cows Mowing: mowing once a year in September

Table 2 Arithmetic mean, standard deviation (SD), and minimum (Min) and maximum (Max) values for the landscape metrics and diversity response variables analysed.

Variable	Mean	SD	Min	Max
Altitude (m a.s.l.)	1111	170	800	1440
Patch size (ha)	10.58	12.85	0.87	67.27
Nearest Distance (ND; m)	656.1	476.27	134.6	2657
Buffer-1km (number of neighbouring fens)	8.38	7.94	0	29
Buffer-2km (number of neighbouring fens)	22.92	14.33	0	55
NN-Area (ha)	2.91	2.29	0.58	12.65
Shannon index vascular plants 1995	3.69	0.17	3.37	3.98
Shannon index vascular plants 2005/06	3.72	0.27	3.18	4.14
Simpson index vascular plants 1995	0.96	0.009	0.93	0.97
Simpson index vascular plants 2005/06	0.96	0.012	0.91	0.98
Habitat specialist density vascular plants 1995	13.25	3.28	7	19
Habitat specialist density vascular plants 2005/06	12.44	3.35	6	18
Total species density vascular plants 1995	60.44	9.19	43	80
Total species density vascular plants 2005/06	63.22	9.83	41	86
Bryophyte species density 1997	33.00	6.47	23	48
Bryophyte species density 2005/06	36.44	8.18	20	55

Table 3 The multivariate models for the response variables in each census (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, (*) $p < 0.1$); interaction terms are shown by × signs (e.g., ND × altcl).

	Vascular plants										**Bryophytes**			
Factors	Specialist species density/site 1995	Specialist species density/site 2005/06	Non-specialist species density/site 1995	Non- specialist species density/site 2005/06	Total species density/site 1995	Total species density/site 2005/06	Shannon 1995	Shannon 2005/06	Simpson 1995	Simpson 2005/06	Total species density/site 1997	Total species density/site 2005/06	Specialist species density/site 1997	Specialist species density/site 2005/06
Management (Man)			**0.01***	**0.003****	**0.005****	**< 0.001*****	**0.005****	**< 0.001*****		**0.01***	**0.001****			
Area [m^2]		**0.02***		0.08 (*)	**0.04***	**0.01***	0.05 (*)	**0.03***	0.06 (*)					
Obstacles	**0.001****	**0.06***			**0.03***		0.08 (*)					**0.03***		
Log nearest distance ND [m]	**0.01***	**0.02***		**0.03***	**0.03***	**0.005****	0.05 (*)	**< 0.001*****		**< 0.001*****				
Nearest neighbour area				**0.02***		**0.03***		**0.002****		**0.01***				
Number of habitat neighbours (Buffer-1 km)	**< 0.001*****	**0.006****												
Number of habitat neighbours (Buffer-2 km)	**0.002****	**0.01***												
Log ND × Buffer-1km		**0.04***									**0.03***			
Log ND × Buffer-2km														
Altitudinal class (altcl)	**0.001****	**0.01***								**0.02***				
ND × altcl	**0.01***								**0.04***					
Man × altcl	**0.008****	0.06 (*)	**0.01***	**0.007****	**0.003****	**0.007****	**0.01***	**0.001****	0.07(*)	**0.005****	**0.01***	**0.002****		
Max. annual degree days (MaxD)	0.09 (*)							**0.02***						
July mean precipitation sum		0.08 (*)									**0.03***			
Man x MaxD							0.09 (*)		**0.005****	**0.03***				

Table 4 Results of repeated-measures analyses. All interactive effects with census date were tested, but only significant effects are listed. Dependent variables were assessed on a total area of 10 m^2 (five 2 × 1 m plots) per site; (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, (*) $p < 0.1$).

	Vascular plants					**Bryophytes**	
Factors	Specialist species density	Non-specialist species density	Total species density/site	Shannon	Simpson	Total species density	Specialist species density
Management (Man)	0.08 (*)	**0.005****	**< 0.001*****	**< 0.001*****	**0.02***	**0.01***	
Area [m^2]	**0.02 ***	0.09 (*)	**0.01***	**0.01***	0.09 (*)		
Obstacles	**0.004****		**0.02***	0.09 (*)			
Log nearest distance ND [m]	**0.006****		**0.002****	**0.002****	**0.008****		
Nearest neighbour area	0.08 (*)	0.05 (*)		**0.01***	0.05 (*)		
Number of habitat neighbours (Buffer 1 km)	**< 0.001*****						
Number of habitat neighbours (Buffer 2 km)	**0.001****						
Log ND × Buffer 1 km	**0.04***						
Log ND × Buffer 2 km	_						
Altitudinal class (altcl)	**0.004****						
ND ×altcl	0.07 (*)						
Man ×altcl	**0.03***	0.08 (*)	**< 0.001*****	**< 0.001*****	**0.04***	**0.002****	
Max. annual degree days (MaxD)							
July mean precipitation sum	**0.03***						
Srad					**0.04***		
Man × MaxD							
Census	**0.02***	**0.001****	**0.01***			**0.004****	
Log ND x census					**0.003****		
Obstacles x census						0.07 (*)	
N.area x census				**0.04***			
Nearest dist × Buffer 2 km x census			**0.03***	**0.04***	**0.007****		
Nearest dist × Buffer1 km x census						**0.03***	
Altcl x census					**0.009****		
MaxD x census				**0.02***	**0.009****		
Srad x census					**0.02***		

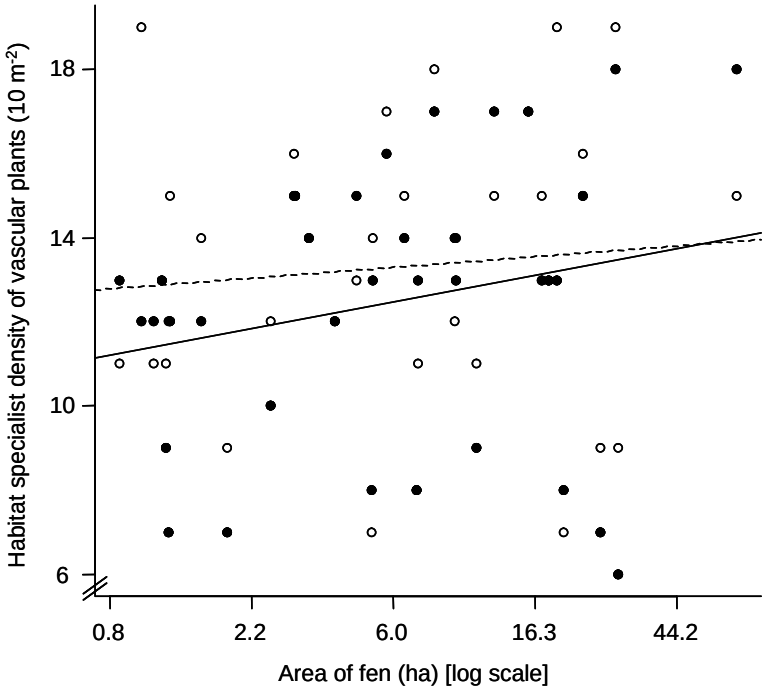


Fig. 1 Relationship between vascular plant habitat specialists density and patch size for both surveys, white points, dotted line: 1995 (*ns*); black points, solid line: 2005/06 (*P*= 0.02).

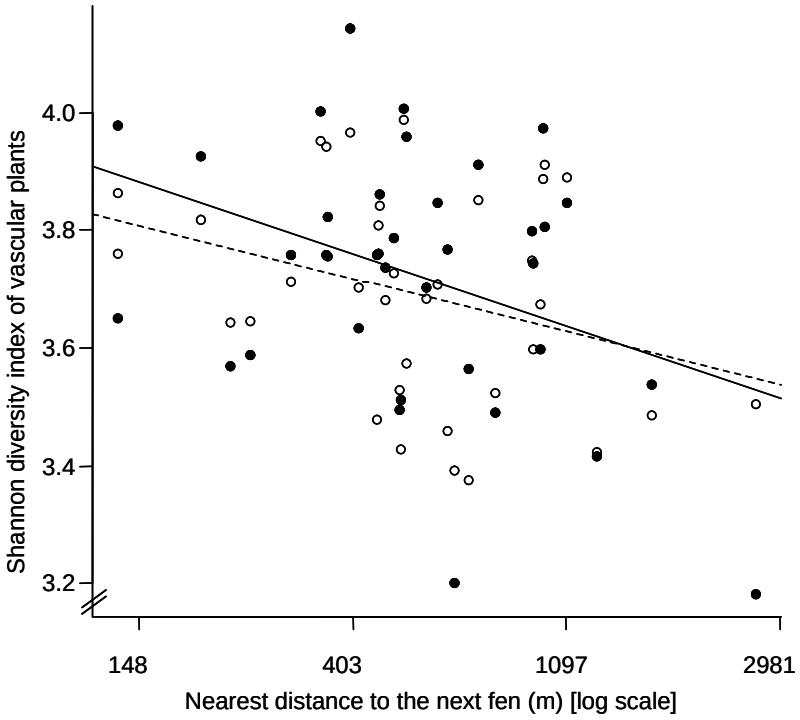


Fig. 2 Relationship between vascular plant species Shannon diversity index and nearest distance to the next fen for both surveys: white points, dotted line: 1995 (P= 0.05); black points, solid line: 2005/06 ($P< 0.001$).

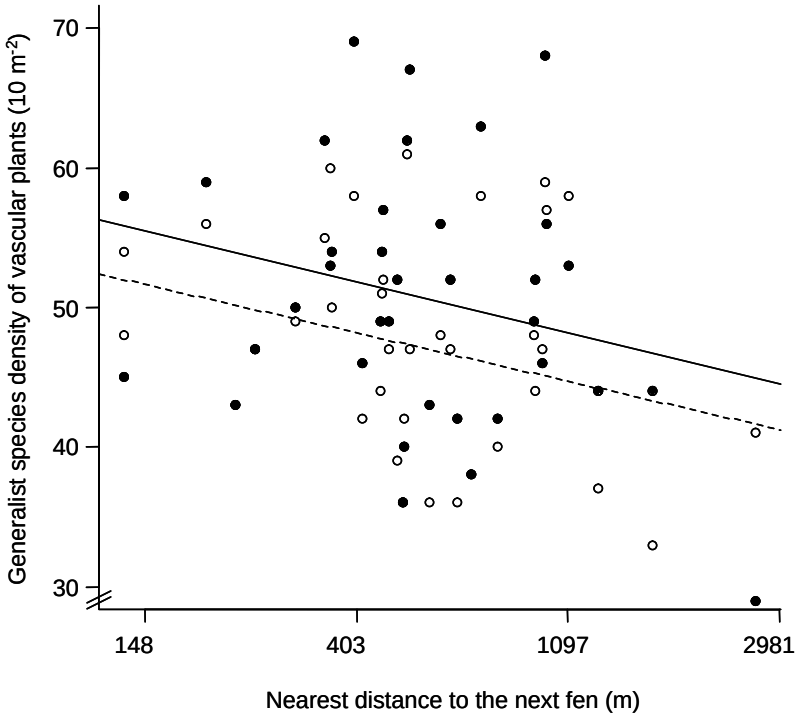


Fig. 3 Relationship between vascular plant generalist species density and nearest distance to the next fen, for both surveys: white points, dotted line: 1995 (*ns*); black points, solid line: 2005/06 (P= 0.03).

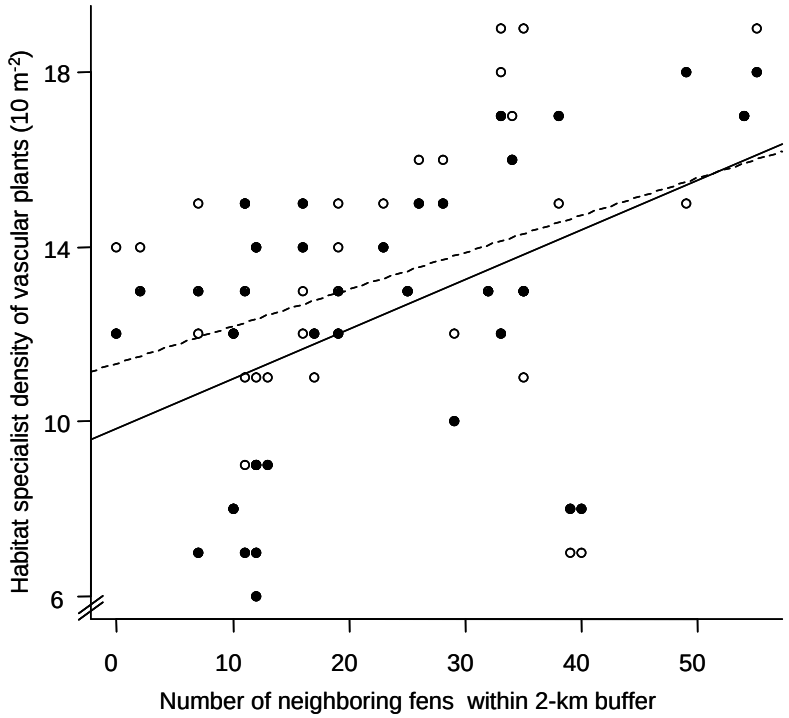


Fig. 4 Relationship between vascular plant habitat specialists density and number of neighbouring fens within a 2-km buffer around the focal fen for both surveys: white points, dotted line: 1995 (P = 0.002); black points, solid line: 2005/06 (P = 0.01).

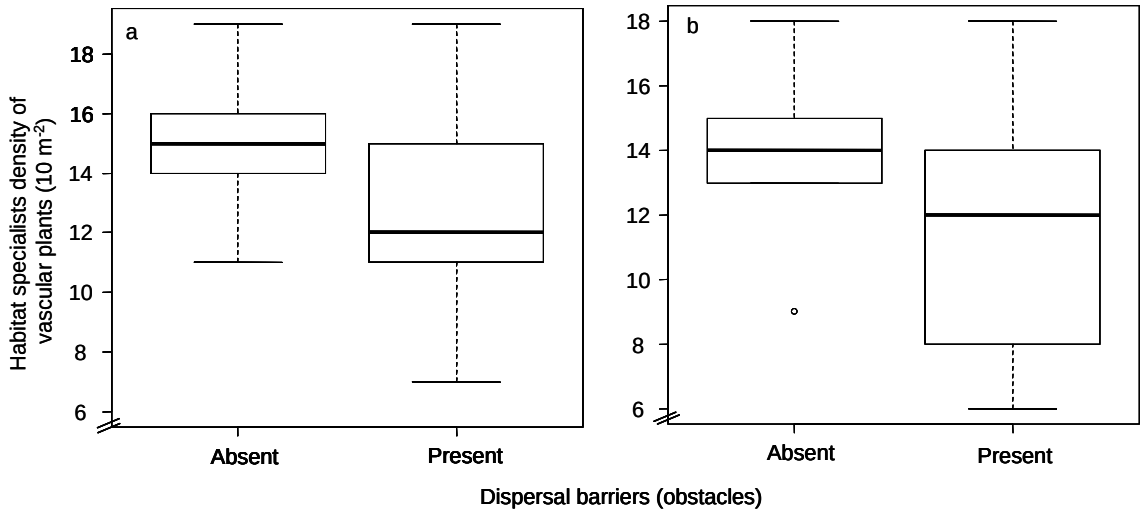


Fig. 5 Effect of obstacles to dispersal on vascular plant habitat specialists density per site (10 m^2): a) 1995 (P = 0.001), b) 2005/06 (P = 0.06). Bold horizontal lines show the median, boxes show the interquartile range, and the whiskers show the maximum and minimum values.

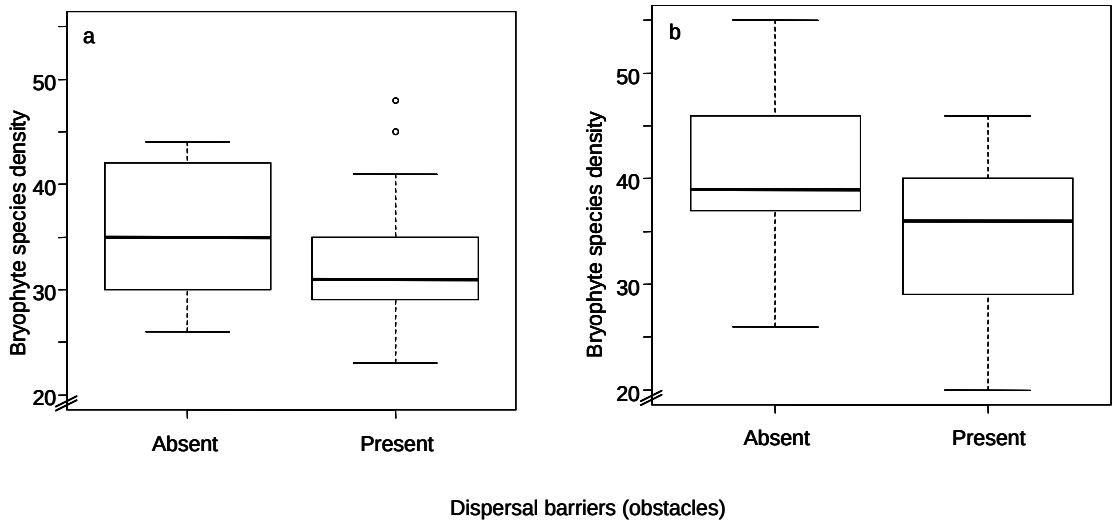


Fig. 6 Effect of obstacles to dispersal on bryophytes species density per site (10 m^2):

a) 1997 (*ns*), b) 2005/06 (*P*= 0.03). Bold horizontal lines show the median, boxes show the interquartile range, and the whiskers show the maximum and minimum values.

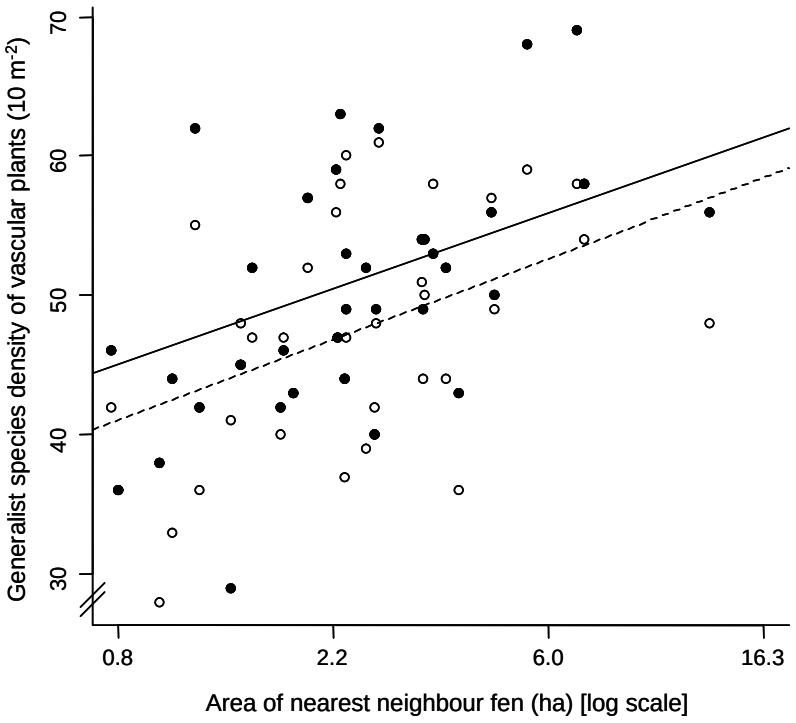
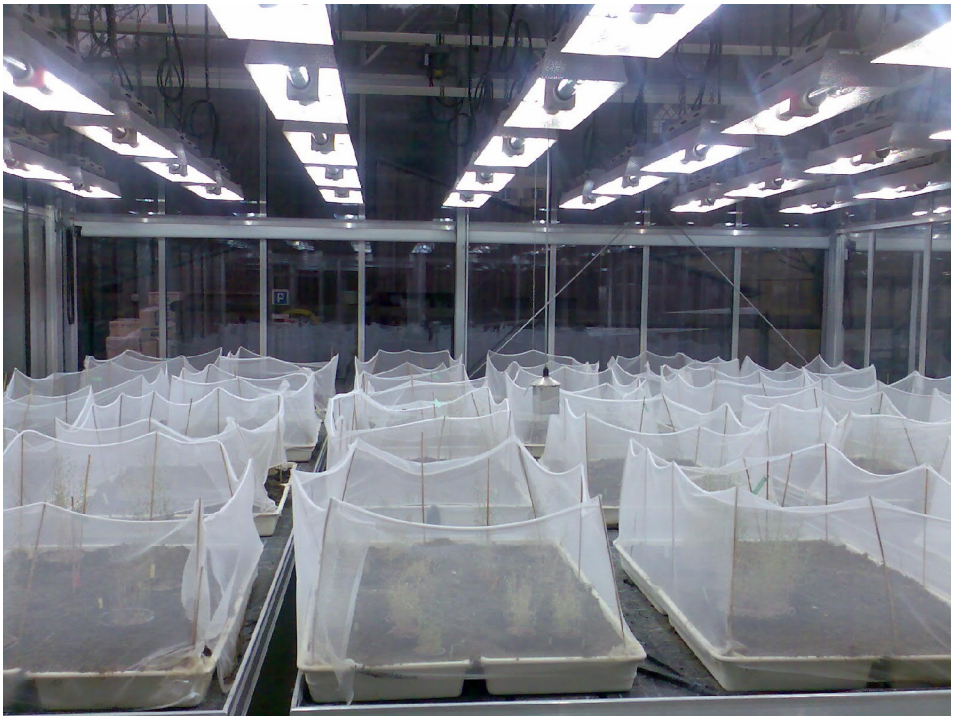


Fig. 7 Relationship between vascular plant generalist species density and nearest neighbour area for both surveys: white points, dotted line: 1995 (ns); black points, solid line: 2005/06 (*P* = 0.02*).

Chapter 4

A multi-generational landscape experiment with *Arabidopsis thaliana*. 1: Landscape-level effects

Sima Fakheran, Cloé Paul-Victor and Lindsay A. Turnbull



ABSTRACT

We studied multi-generational experimental metapopulations of *Arabidopsis thaliana*. We created artificial landscapes with islands of suitable habitat embedded in an unsuitable matrix to simulate habitats in nature. We manipulated both the degree of fragmentation by using four patch sizes, and the rate of patch disturbance by using two different patch disturbance regimes, such that patches either remain in place (static) or were destroyed and re-created in new locations (dynamic). The number of seedlings in each patch, the number of surviving adults, and the total biomass were measured in each generation. These variables were analysed using linear mixed-effects models, considering landscape identity as a random effect. Patch disturbance had a strong effect on population density with much higher numbers of seedlings in static than in dynamic landscapes. These differences in density in turn caused differences in the survival of seedlings to adulthood, with seedlings experiencing much higher death rates in static than in dynamic landscapes. We also found a positive relationship between total biomass and density of the surviving adults. The landscape fragmentation treatment (the size and number of suitable habitat patches) had less impact and was often highly variable in its effect among generations. The fact that at least one of our experimental landscape treatments had such a dramatic effect strengthens our conclusions that patch dynamics are likely to be a potent force in the evolution of annual plant life-histories.

INTRODUCTION

Human activities have been fragmenting natural habitats into fewer and smaller patches at an accelerating pace (e.g. Saunders et al., 1991). In addition, these small habitat fragments are increasingly altered or completely destroyed by human and natural disturbances (Caley et al., 2001, Casagrandi & Gatto 2002). Fragmentation and disturbance are likely to occur together and may also interact (Fahrig 1997; Casagrandi & Gatto 2002).

Although habitat fragmentation is usually accompanied by habitat loss (Andren 1994 ; Fahrig 1997, Parker & Mac Nally 2002), many species naturally occur in fragmented landscapes or metapopulations (Levins & Culver 1971; Hanski 1983; Hanski 1999). In many plant species populations are patchily distributed, occupying areas of suitable habitat scattered within an inhospitable matrix (Groom & Schumaker 1993; Hanski 1999; Cook et al., 2002). For example, annual plants often rely on small gaps free from competition with perennials, which are frequently created by disturbances of the perennial matrix (Carey & Watkinson 1993; Turnbull et al., 2007). Depending on how such suitable gaps are created, these patches could be highly persistent through space and time or be highly dynamic. For example, if summer drought creates gaps, it is more likely to remove perennial cover and hence create an opportunity for annuals in places where soils are thin; thus, suitable patches may occur in the same places each year. In contrast, if animal movement such as trampling creates the suitable gaps, then gaps may be short-lived and occur unpredictably in space (Grubb 1986). Hence, suitable patches may be quite dynamic (Turner et al., 2001).

The pattern of gap creation will only be important in populations with local as opposed to global dispersal. If plants already disperse their seeds globally in a landscape, such that there is no relationship between maternal plants and seeds, then seeds can disperse equally well to new patches as to existing patches (Hamilton & May 1977; Gros et al., 2006). However, with local dispersal, where we can expect to find seeds close to the maternal plants, then most seeds will inevitably fall within existing patches. In this case, changing the patch

disturbance rate will have profound impacts on population dynamics, e.g. typical densities and on dispersal traits such as seed size and height (Roy et al., 2004).

Patch dynamics are important for annual plants because the adults die each year and seeds must colonise suitable patches to maintain the population. Colonization is a process which begins with dispersal (Richards 2000) and increases if plants produce more seeds and disperse them over longer distances, so that the chance that a species persists in a landscape depends both on the habitat configuration and the species' dispersal ability (Gardner et al., 1987; Kindlmann et al., 2005; O'Neill et al., 1988; With & Crist 1995). If suitable patches remain in the same place year after year then limited dispersal is likely to be favoured (Hastings 1983). However, if suitable patches move around then good dispersal will be selected for. Traits likely to affect dispersal ability include plant height and branching pattern as well as seed size. For example, taller plants are expected to disperse seeds further as might plants with more branches (Thiede & Augspurger 1996; Wender et al., 2005). Small seed mass is also often associated with enhanced dispersal because small-seeded plants tend to produce more seeds (Nathan et al., 2002; Wender et al., 2005). For example, if there is a trade-off between seed size and number, small-seeded individuals produce more seeds (Smith & Fretwell, 1974) and hence have a higher chance of colonizing new patches**.** They may also travel further especially if the species is wind-dispersed (Soons & Bullock 2008).

The typical patch size as well as disturbance rate is also likely to be important. If we take a continuous suitable area and fragment that into several smaller pieces then we might also expect consequences both for population dynamics and trait evolution. For example, small patches might be more vulnerable to desiccation, while large patches more attractive to herbivores (Sheehan & Shelton 1989; Faveri et al., 2008). Patch size also affects the number of intraspecific competitions that individuals typically interact with.

Our experiment

Here we present an experimental study in which we manipulate landscape characteristics for populations of the annual plant *Arabidopsis thaliana. Arabidopsis thaliana* (L.) Heynh., wall cress or mouse-ear cress, typically displays a winter annual or spring annual life history (Nordborg & Bergelson 1999; Wender et al., 2005*)*. It is native to Western Eurasia and is now naturalized in North America, Asia, Europe and North Africa (Al-shehbaz & O`Kane, 2002; Hoffman 2002). *A. thaliana* is autogamous and has a high rate of self-fertilization (< 0.3 % outcrossing; Abbott & Gomes 1989), meaning that there is very little recombination.

We created artificial landscapes with islands of suitable habitat embedded in an unsuitable matrix to simulate islands of natural habitats in nature (Hanski 1999; Cook et al., 2002). We manipulated both the degree of fragmentation by using four patch sizes and the rate of patch disturbance by using two different patch disturbance regimes (static vs. dynamic). In static landscapes, suitable habitats remain in the same place over several generations; but in dynamic landscapes suitable habitat patches are only available for one generation. They are then destroyed and regenerated in new locations in the landscape (for more details see Methods, *Dynamic vs. static Landscapes*)**.** In this chapter, we consider the effect of our landscape manipulations on population characteristics which we did not deliberately manipulate such as density of seedlings, survival of plants to adulthood and total biomass.

The following questions are specifically addressed*:*

1) How do patch size and patch disturbance rate affect population density?

2) How do patch size and patch disturbance rate affect survival of seedlings to adulthood?

3) How do patch size and patch disturbance rate affect total biomass?

4) Do the spatial effects vary depending on whether the landscape is static or dynamic, i.e. are there interactions between our two treatments?

MATERIAL AND METHODS

Biological Material

We selected a population of 162 recombinant inbred lines (RILs) of *Arabidopsis thaliana* (Alonso-Blanco et al., 1999). The RILs are derived from reciprocal crosses between the two pure lines the small-seeded Landsberg *ERECTA* (L*er*) obtained as a mutant (*er*) from an accession of northern Europe (Rédei 1962; Rédei 1992), and the large-seeded Cvi, an accession from the tropical Cape Verde Islands (Lobin 1983). We selected 17 RILs from the possible 162 plus the two parent lines to use in the landscape experiment described here (see Table 1). We selected these lines in such a way as to maintain the seed mass variation present in the original RIL population (1.45 - 3.73 mg per 100 seeds). The lines can inherit the mutation *ERECTA* from the L*er* parent. Ten of the selected lines carry this mutation, the other nine not (For more details see: Chapter 4, *Biological materials*). Lines carrying the *ERECTA* mutation typically have short and upright stems, round leaves, short petioles and pedicels, flowers clustered at the top of the inflorescence, short and wide siliques with blunt tips, a compact inflorescence and reduced height (TAIR). The reduced height is the most striking thing about plants carrying the *ERECTA* mutation. Thus plants carrying this mutation are expected to achieve poorer spatial dispersal of their seeds. All seeds were obtained from The Arabidopsis Information Resource (TAIR).

Landscapes

We set up a habitat fragmentation experiment using 24 landscapes with different degrees of fragmentation of the suitable habitat. The experiment was run for five generations in a glasshouse. After five generations individuals were sampled from each landscape and grown individually in a common garden experiment. Each landscape measured 90 × 64 cm, and consisted of patches in which *Arabidopsis* plants were allowed to grow (suitable habitat) and

the matrix in which any plants growing were regularly removed (unsuitable habitat).The suitable habitat made up around 7 % of the total landscape (see Table 2).

Landscapes were constructed by filling a large tray (90 × 64 cm) with a mix of 50% soil and 50% sand. The patches were cylindrical slices of PVC tubing, cut to the same depth as the trays (70 mm). Patches were pushed into the soil so that their tops were level with the soil surface. The suitable habitat consisted of 2, 4, 8 or 16 patches (Fig. 1). The patch size was chosen to keep the total area of suitable habitat constant. However, due to constraint of available material, the total area of suitable habitat varies slightly with the number of patches (Table 2). The four patch sizes provide different degrees of habitat fragmentation. There were six replicates of each level of habitat fragmentation making 24 landscapes in total. Patches were located within landscapes in a stratified random way. The landscapes were divided into four equally-sized quarters and patches were located in the following way: in 2-patch landscapes, only one patch was allowed in each of two randomly selected quarters, in 4-patch landscapes only one patch was allowed per quarter, in 8-patch landscapes two patches were allowed per quarter, and in 16- patch landscapes four patches were allowed per quarter; however the location of patches within quarters in all cases were selected at random. This minimised within- treatment variations.

Dynamic vs. static Landscapes

As well as the patch size treatments, we imposed two different patch disturbance regimes. In the first (which we call static) seeds which fall into the natal patch are returned to the surface of a new patch in the next generation. To do this, seeds were manually released from the siliques (by gently shaking the plants by hand) once the plants were mature and seeds were ripe. All plant material was then measured and weighed (see *Data collection*). The surface layer of soil containing seeds was then scraped away from each patch and placed in a Petri dish (one per patch) and placed in a fridge for one week. During this time all remaining soil

was removed from the existing patches and replaced with fresh soil made up in the same way as before (50% soil and 50% sand). Thus seeds which do not disperse away from their natal patch have a much higher chance of entering the next generation, although seeds which land in another patch, and not in the matrix, can also enter the next generation.

In the second patch disturbance regime (which we call dynamic) new Petri dishes (of the same number and size as the existing patches) were randomly placed around the landscape to collect dispersing seeds before plants began to flower (See Fig. 2). Seeds were then manually released from siliques in exactly the same way as for static landscapes. The new Petri dishes containing any dispersed seeds were removed and placed in the fridge for one week. All plants were removed and weighed and all patches refilled with fresh soil. Thus in the dynamic landscapes, seeds falling back into the natal patch have no chance of entering the next generation. Seeds from static and dynamic landscapes were removed at the same time and placed in the same fridge together for the same length of time. Notice that, in static landscapes each patch maintains its identity through time (seeds taken from patch *i* are returned to the same patch *i*, but in dynamic landscapes a patch in generation $t+1$ can not be identified with any particular patch in generation *t*.

Initialising landscapes

In generation 1, landscapes were initiated by introducing seeds of each of the 19 lines in the following way. The 19 selected lines were counted and sown so as to obtain equal initial densities in landscapes containing different patch sizes. We sowed one seed per line into each of the smallest patches, and 2, 4 and 8 seeds per line into each patch in the 8, 4 and 2-patch landscapes, respectively. Thus initially 16 seeds of each line were introduced into each landscape. Seeds were initially counted into eppendorf tubes and then kept in a cold room at 4 °C for one week to overcome seed dormancy and ensure uniform germination of different

lines. In total 7296 seeds were sown in the first generation in 180 patches of our 24 landscapes.

Timetable

In the first week after sowing we sprayed water on the soil every day to encourage and synchronise germination. After the first week plants were irrigated by flooding the table twice a day with water. After germination, the number of seedlings per patch was recorded for each landscape two times (10 and 17 days after sowing the seeds). When the plants began to produce fruits (around 25 days after sowing the seeds), we put netting around each landscape to avoid seeds dispersing among landscapes and put out new Petri dishes in dynamic landscapes to collect dispersing seeds for the next generation.

We cut off watering after 49 days but allowed plants to continue growing for a further 14 days. After eight weeks, when most siliques were mature, we collected seeds from a sample of plants for weighing (see Chapter 5). After nine weeks, when most of the siliques had dehisced and most of the seeds were dispersed, we removed all plants and measured the height and number of branches of 50% of the individuals in each landscape (see Chapter 5). The above-ground parts of the plants were dried at 80 °C for 48 h to determine dry weight biomass. Petri dishes containing dispersed seeds were removed from dynamic landscapes and the soil surface from each patch was scraped away in static landscapes. All seeds were then refrigerated for at least one week. Seeds of the first generation were sown in October 2006 and the experiment ended in January 2008. Then, seeds from plants belonging to the fifth generation were sampled to grow up in standardized conditions (for more details, see Chapter 5). This final stage of experiment ended in April 2008.

In the glasshouse, we kept a minimum daytime temperature (22°C) and a minimum night time temperature (20°C) .There was additional lightening which came on when natural light fell below 25 klux and hence we ensured a 16 h day in all generations. However,

conditions in the glasshouse were variable between generations. As the glasshouse had no cooling system, in May 2007 the temperature in glasshouse rose to 38°C, and therefore in generation 3, seedling survival to adulthood was very low. Then, to avoid heat stress again, we put the seeds of the third generation in fridge till September when generation 4 was initiated.

To reduce damage to plants by Sciarid flies (e.g. *Bradysia pavpera)* which feed on roots and rosettes, the insecticide Thiamethoxam (*Actara G.*) was applied at 1% concentration in granular form in week 2. However, it was never 100% successful and some flies were observed in each generation.

Data collection

The following variables analysed in this chapter were measured at the same time point in each generation.

1) *Number of seedlings emerging in each patch* (10 and 17 days after sowing).
2) *Final number of surviving adults* (week 9).
3) *Total biomass of final surviving adults* (week 9).

Statistical analysis

Here we present the analysis of number of seedlings, seedling survival to adulthood, and total biomass. These are landscape characteristics which we did not deliberately manipulate but which nevertheless changed as a consequence of our experimental treatments. The total biomass and the total number of seedlings were analysed using linear mixed effect models, using the function lme in the stats package R (Pinheiro & Bates 2000; R Development Core Team 2007). Landscape identity (i.e. 1-24) was treated as a random effect and formed the error term for the landscape-level treatments: patch size and patch disturbance regime (static vs. dynamic) and their interaction.

For the survival of the seedlings to adulthood we calculated the ratio log [number of adults per patch / number of seedlings per patch]. This ratio takes the value zero (0) when all seedlings survive and is positive when there are more adults than seedlings. This did occur in some patches in dynamic landscapes and indicates further recruitment after our seedling census. Normally the ratio is negative because more seedlings died than were recruited. This analysis was carried out at the patch level rather than the landscape level, because local rather than landscape processes should be more influential in determining survival rates. Patch identity was treated as a random effect nested within landscape identity.

RESULTS

Total number of seedlings

The total number of seedlings emerging after 10 days is shown in Fig. 3 by disturbance regime and in Fig. 4 by patch size. The number of seedlings was much higher in generations 2-5 than in generation 1, when we introduced a small number of seeds by hand (see Methods, *Initialising landscapes*). Because we included generation 1 in this analysis, we fitted generation as a factor with 5 levels. Generation was highly significant (Table.3). The most likely causes of the variation among generations 2-5 are environmental differences; for example, temperature (see Methods, *Timetable*). This probably affected germination and survival rates due to desiccation of seedlings and the number of Sciarid flies emerging (e.g. *Bradysia pavpera)* which attacked the roots and rosette leaves etc.

Both disturbance regime and patch size have pronounced interactions with generation (Table. 3). For the disturbance regime there was very little difference in the number of seedlings found in static vs. dynamic landscapes in generations 1 and 2; however in generations 3, 4 and 5 there were many more seedlings in static than in dynamic landscapes (Fig. 3). For patch size, there was a negative relationship between patch area and number of seedlings in generations 1 and 2; however there was a slightly positive relationship in

generations 3, 4 and 5. The covariate total area of suitable habitat was not significant ($F_{1,19}$ = 0.499, p = 0.49) indicating that the variation in total suitable area available in landscapes with different patch size did not affect the number of seedlings emerging.

Survival of seedlings to adults

The survival of seedlings from day 10 to adulthood is presented in Fig. 5 by disturbance regime and by patch size in Fig. 6. Again there is a little evidence of a consistent trend with time and so generation was fitted as a factor with five levels. It is immediately clear that survival of seedlings to adulthood was always poorer in static compared with dynamic landscapes (Fig. 5). The statistical analysis also indicates that, although the interaction of disturbance regime with generation is significant (Table. 4), it is only the strength of the effect and not its direction that varies among generations. In contrast, the effect of patch size on seedling survival changes its direction among generations: there was a positive relationship between patch area and survival of seedlings in generations 1 and 2; however there was a slightly negative relationship in generations 3, 4 and 5.

Because of the expected relationship between mortality and density, we performed a second analysis with seedling density fitted first as a covariate. This was highly significant (Table. 5) and its inclusion in the model made the main effect of patch disturbance treatment completely non-significant (Table. 5). Hence the differences in the survival of seedlings in static vs. dynamic landscapes are entirely due to differences in density. The interaction with generation remains but it is weak. Controlling for density, however, did not affect the significance of generation (Table. 5). Hence, generational effects on seedling survival are not due to differences in density. They are therefore more likely due to differences in temperature or densities of Sciarid flies. After controlling for seedling density there was a large and significant main effect of patch size on seedling survival (Table. 5). This is positive, such that seedlings survive better in large patches. Although, there is a significant generation × patch

size interaction, this is weak compared to the main effect because it is only the strength and not the direction of the effect which varies between generations. This positive effect of patch area on seedling survival is only apparent once the differences in seedling density are controlled for (Table. 4 vs. Table. 5).

Total biomass

Total biomass in each generation is plotted by disturbance regime (Fig. 7) and by patch size (Fig. 8). It is immediately clear that biomass was unusually high in generation 2 and shows no particular trend with time. Hence we did not fit generation as a continuous variable although it was again highly significant (Table. 6). The graphs show and the analysis confirms that the effect of both disturbance regime and patch size also vary with generation (generation × patch size, $F_{4,80} = 17.1$, $p < 0.0001$; generation × disturbance, $F_{4,80} = 9.00$, $p < 0.0001$). There was a negative relationship between patch size and total biomass in generations 1 and 4 but, a strong positive relationship in generation 2. In generations 3 and 5 there was little relationship between total biomass and patch size. The intercept of the patch area relationship was higher in static landscapes in generations 2, 3 and 4, but lower in generation 1 and 5.

We also fitted two covariates, total suitable area, which varies slightly with patch area (see Table. 7), and the total number of surviving adults. Both of the covariates were log transformed because they are not expected to have a linear effect. Total area has a significant effect ($F_{1,19} = 11.02$, $p = 0.004$), although surprisingly there was a negative relationship between total biomass and total area; however there was a positive relationship between total biomass and the number of surviving adults ($F_{1,79} = 91.06$, $p < 0.0001$). The positive relationship between adult density and yield among landscapes indicates that in some landscapes densities are below carrying capacity. After fitting the two covariates, the interactions generation × patch size and generation × patch disturbance remain significant (Table. 7).

DISCUSSION

We created artificial fragmented landscapes consisting of patches of suitable habitat within an inhospitable matrix, initially seeded with nineteen RILs of *Arabidopsis thaliana* varying in their seed mass and height. The landscapes were allowed to self-seed and evolve for five generations. The experimental treatment patch disturbance, in which patches either remained in place (static) or were destroyed and re-created in new locations (dynamic), had a dramatic effect on population density. Differences in density in turn, caused differences in the survival rate of seedlings to adulthood, with seedlings experiencing much higher death rates in static than in dynamic landscapes. The negative effects of density on the individual performance and survival of *Arabidopsis thaliana* is already well known (Myerscough & Marshal 1973; Mosleh Arani 2005). Our second landscape-level treatment, fragmentation (the size and number of suitable patches), had less impact and was often highly variable in its effects on density, survival and biomass among generations. The surprisingly small effects of fragmentation are perhaps because the total area was approximately constant, while in nature fragmentation is usually linked to a decrease in the total area of suitable habitat (Fahrig 1997). In addition, although landscapes with two large patches have a lower edge/area ratio and might therefore lose fewer seeds to the matrix, they also have fewer patches in total. Thus dispersing seeds are less likely to land in another suitable patch. These two effects, which work in opposite directions, might have counteracted each other to some extent. However, patch size had a large and positive effect on seedling survival once differences in seedling density were controlled for, indicating that increasing fragmentation might eventually lead to lower population density.

As outlined in the introduction, differences in seedling density between static and dynamic landscapes will only occur with local rather than global dispersal. As we observed no difference in seedling density between static and dynamic landscapes in generations 1 and 2, it is probable that generation 1 plants did achieve approximately global dispersal. This is

probably because plants were at low density and hence taller (see Chapter 5) and importantly, much more heavily branched than in subsequent generations (see chapter 5). Taller plants should inevitably disperse seeds further (Wender et al., 2005), and branching allows plants to release seeds some distance from the centre of the rosette. In subsequent generations the density of seedlings was much higher in static than in dynamic landscapes indicating local dispersal. Thus, once density builds up, dispersal kernels are inevitably altered (Clark et al., 1999; Nathan 2006) and global dispersal is difficult to achieve, even in such small landscapes.

Our study uniquely manipulated features of the landscape rather than deliberately selecting for particular life-history traits, for example by only choosing seeds from the tallest individuals. Interestingly, patch-level disturbance has several consequences for population dynamics and structure. First, highly disturbed landscapes will inevitably directly select for dispersal traits. However, if highly disturbed landscapes also typically have lower densities, then traits associated with high competitive ability may also have less adaptive value. Similarly, because of the increased survival from seedling to adulthood, traits which increase the probability of survival will also experience decreased selection. There is also the possibility that higher trophic levels will be affected. For example, if herbivores can feed more efficiently in high density patches, then they too will be affected by such landscape-level processes (Tscharntke & Brandl 2004).There has also been recent interest in the kin structure of *Arabidopsis thaliana* (Weinig et al., 2007). For example, in a static landscape, most of an individual's immediate neighbours may actually be genetically identical as non-dispersing seeds accumulate in natal patches. However, in a more highly disturbed landscape, patches will typically contain a much greater mixture of genotypes as they are colonised by immigrant seeds from around the landscape (Donohue 1997). Thus the kin structure of the landscape will be largely determined by the disturbance rate and the dispersal abilities of its occupants (Jansen & Vitalis 2007).

Our study did not control every aspect of the environment in order to make every generation exactly the same, for example, we did not carefully control the temperature and humidity. Thus there was great variability between generations in seedling densities, survival and biomass which we cannot adequately explain. However, we believe that this variation brings this study a little closer to the natural situation in which years inevitably vary in many different ways. The fact that at least one of our experimental treatments had such a dramatic effect, despite this variation, strengthens our conclusions that patch dynamics are likely to be a potent force in the evolution of annual plant life-histories.

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Table 1 Information about the 19 lines selected for the study. The two accessions L*er* and Cvi are the parents. The 17 remaining recombinant inbred lines (RILs) are derived from reciprocal crosses between the two parents.

NASC	RIL Koornneef	Published Seed Mass (*) [mg]	Sown Seed mass (**) [mg]	*ERECTA* mutation
N8581	L*er*	0.0193	0.0202	1
N8580	Cvi	0.0351	0.0348	0
N22018	CVL19	0.0251	0.0263	1
N22026	CVL27	0.0275	0.0270	1
N22030	CVL31	0.0295	0.0334	0
N22033	CVL34	0.0236	0.0297	0
N22036	CVL37	0.0325	0.0399	0
N22038	CVL39	0.0202	0.0258	0
N22051	CVL53	0.0327	0.0310	1
N22057	CVL60	0.0286	0.0393	1
N22095	CVL125	0.0200	0.0214	0
N22098	CVL128	0.0273	0.0274	0
N22105	CVL135	0.0327	0.0348	1
N22107	CVL137	0.0302	0.0314	0
N22112	CVL142	0.0315	0.0318	1
N22128	CVL158	0.0373	0.0411	1
N22138	CVL168	0.0334	0.0299	0
N22149	CVL179	0.0223	0.0243	1
N22156	CVL187	0.0183	0.0192	1

(*) Source: Alonso-Blanco et al., 1999.

(**) Source of seeds: Arabidopsis center (TAIR).

Table 2 Experimental design: Four different patch sizes provide different degrees of habitat fragmentation. The patch size was chosen to keep the total area of suitable habitat constant; however, it varies slightly with the number of patches.

Number of patches	Area of 1 patch (cm^2)	Total suitable area (cm^2)	% Suitable area
2	240	480	8.3
4	100	400	6.9
8	52	416	7.2
16	25.5	408	7.1

Table 3 ANOVA results of the analysis of total number of seedlings per landscape through generations 1–5.

	numDF	denDF	F-value	p-value
(Intercept)	1	80	678.7055	<.0001
Generation	4	80	112.7155	<.0001
log(Patch. size)	1	20	0.533	0.4738
Disturbance-regime	1	20	8.0151	0.0103
Generation × log(Patch. size)	4	80	10.9737	<.0001
Generation × Disturbance-regime	4	80	33.2051	<.0001
log(Patch. size) × Disturbance-regime	1	20	0.0878	0.7701
Generation × log(Patch. size) × Disturbance-regime	4	80	0.4308	0.786

Table 4 ANOVA results of the analysis of seedlings survival, i.e. log (No. adults/No. seedlings) through generations 1–5.

	numDF	denDF	F-value	p-value
(Intercept)	1	209	384.2654	<.0001
Disturbance-regime	1	20	76.9935	<.0001
Generation	4	209	68.9743	<.0001
log(Patch. size)	1	20	3.9624	0.0604
Disturbance-regime× Generation	4	209	7.2112	<.0001
Disturbance-regime × log (Patch. size)	1	20	0.3984	0.5351
Generation × log (Patch. size)	4	209	6.9988	<.0001
Disturbance-regime × Generation × log(Patch. size)	4	209	2.2829	0.0616

Table 5 ANOVA results of the analysis of seedlings survival, i.e. log (No. adults/No. Seedlings) through generations 1–5, but fitting number of seedlings as covariate.

	numDF	denDF	F-value	p-value
(Intercept)	1	208	447.1698	<.0001
log(No.Seedlings.1)	1	208	557.7832	<.0001
Disturbance-regime	1	20	1.429	0.2459
Generation	4	208	50.3216	<.0001
log(Patch. size)	1	20	73.6682	<.0001
Disturbance-regime × Generation	4	208	3.723	0.006
Disturbance- regime × log(Patch. size)	1	20	0.1074	0.7466
Generation × log(Patch. size)	4	208	4.9454	0.0008
Disturbance-regime × Generation × log(Patch. size)	4	208	1.3303	0.2598

Table 6 ANOVA results of the analysis of total biomass per landscape through generations 1–5.

	numDF	denDF	F-value	p-value
(Intercept)	1	80	174.441	<.0001
Disturbance-regime	1	20	7.91691	0.0107
Generation	4	80	117.6178	<.0001
log (Patch. size)	1	20	7.54275	0.0124
Disturbance-regime × Generation	4	80	9.00378	<.0001
Disturbance-regime × log (Patch. size)	1	20	0.79949	0.3819
Generation × log (Patch. size)	4	80	17.09341	<.0001
Disturbance-regime × Generation × log(Patch. size)	4	80	0.85257	0.4962

Table 7 ANOVA results of the analysis of total biomass per landscape through generations 1–5, but fitting total density, and the total area of suitable habitat, which varies slightly between landscapes with different numbers of patches, as covariates.

	numDF	denDF	F-value	p-value
(Intercept)	1	79	299.79676	<.0001
log(Total. area)	1	19	11.02292	0.0036
log(Total. density)	1	79	91.06835	<.0001
Disturbance-regime	1	19	0.00425	0.9487
Generation	4	79	124.56787	<.0001
log(Patch. size)	1	19	10.77637	0.0039
Disturbance-regime × Generation	4	79	8.38565	<.0001
Disturbance-regime × log(Patch. size)	1	19	0.29141	0.5956
Generation × log(Patch. size)	4	79	8.44641	<.0001
Disturbance-regime × Generation × log(Patch. size)	4	79	0.52649	0.7165

Figure 1 Our landscapes: the suitable habitat consisted of 2, 4, 8 or 16 patches; the four patch sizes provide different degrees of habitat fragmentation. The patch size was chosen to keep the total area of suitable habitat approximately constant.

A) B)

Figure 2 Petri dishes were placed randomly in dynamic landscapes to collect the seeds; (A), The collected seeds from dynamic landscapes (B, right) and soil+ seeds from static landscapes (B, left) were placed in fridge

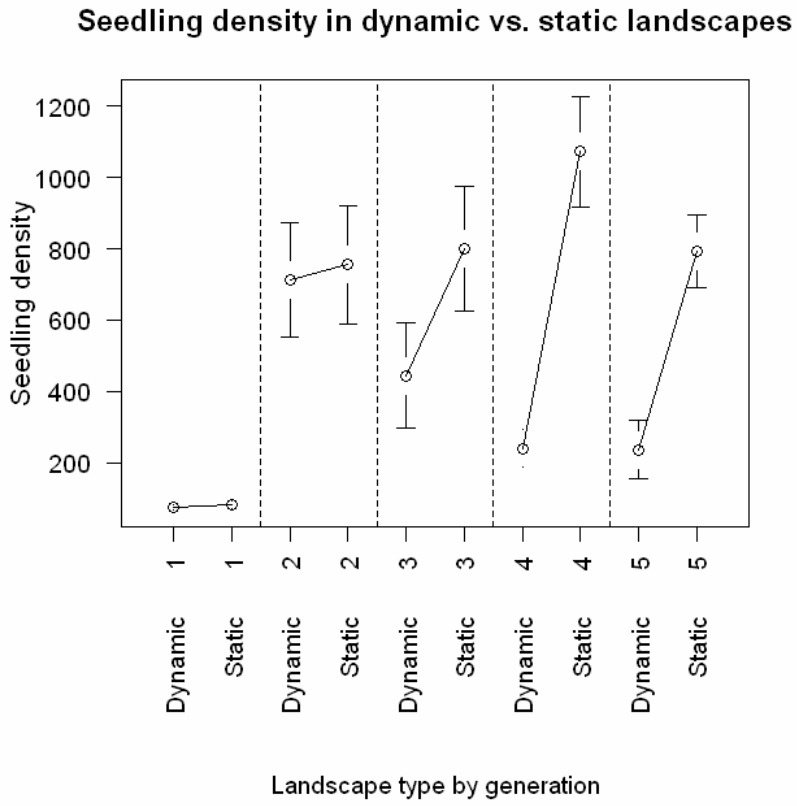


Figure 3 Seedling densities in dynamic vs. static landscape in generations 1–5. The mean and the 95% confidence interval are shown.

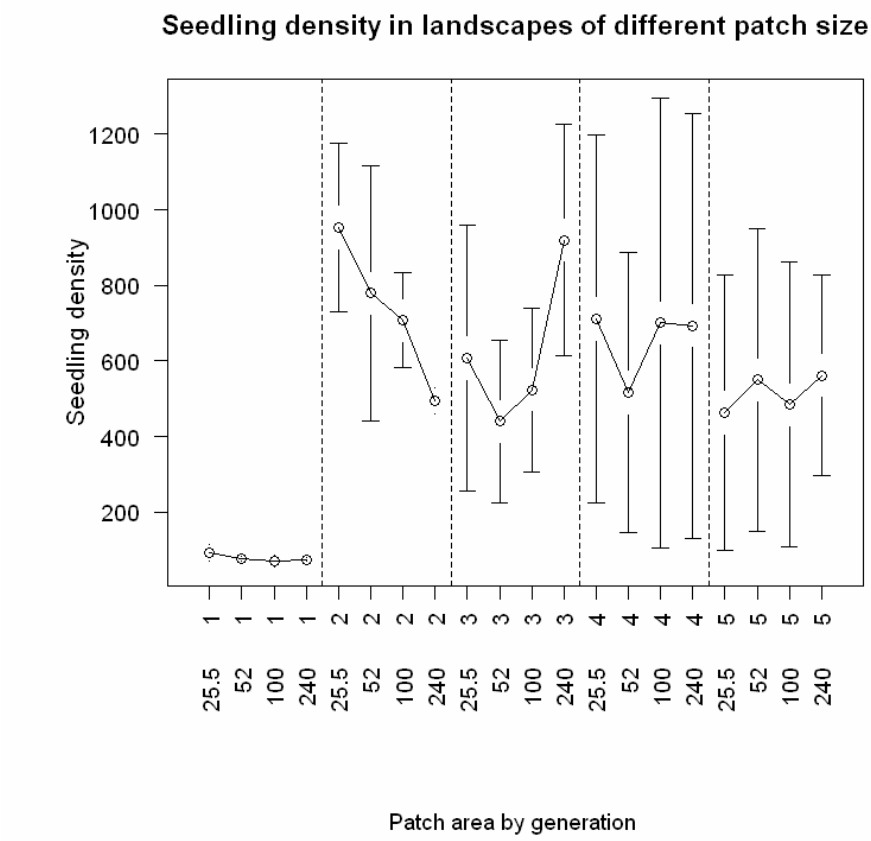


Figure 4 Seedling densities in landscapes of different patch area in generations 1–5. The mean and the 95% confidence interval are shown.

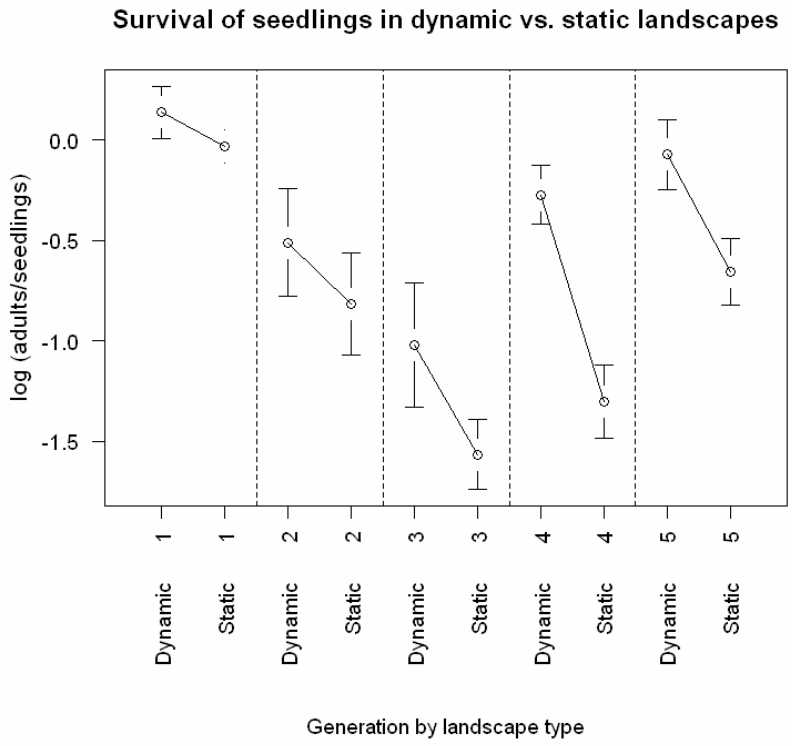


Figure 5 Survival of seedlings in static vs. dynamic landscapes in generations 1–5. The mean and the 95% confidence interval are shown.

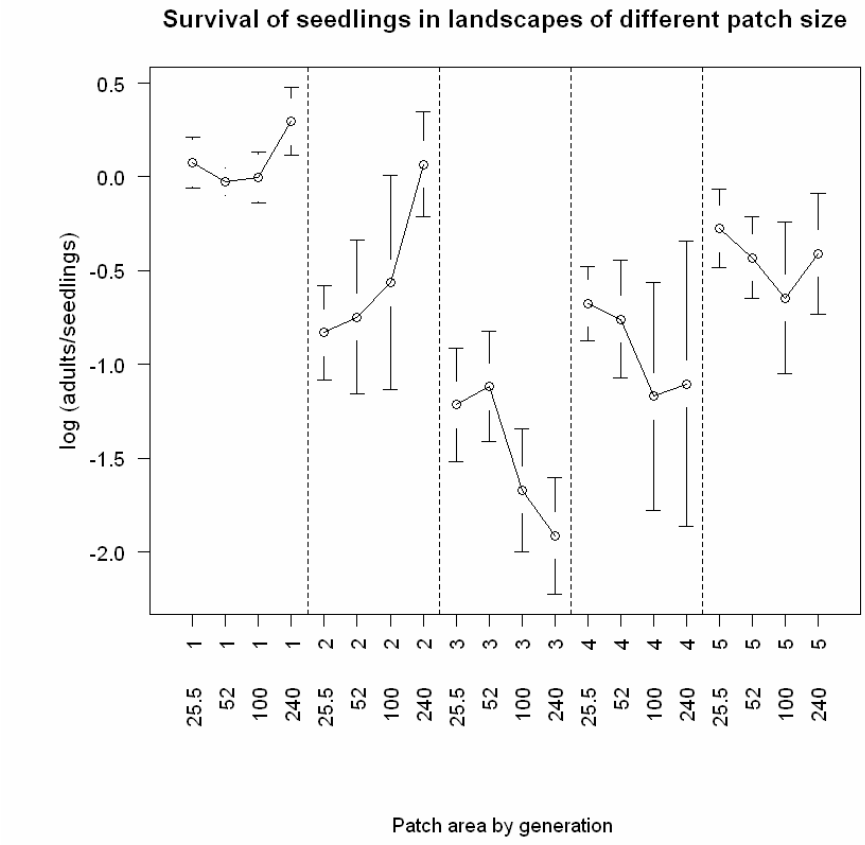


Figure 6 Survival of seedlings in landscape of different patch area in generations 1–5. The mean and the 95% confidence interval are shown.

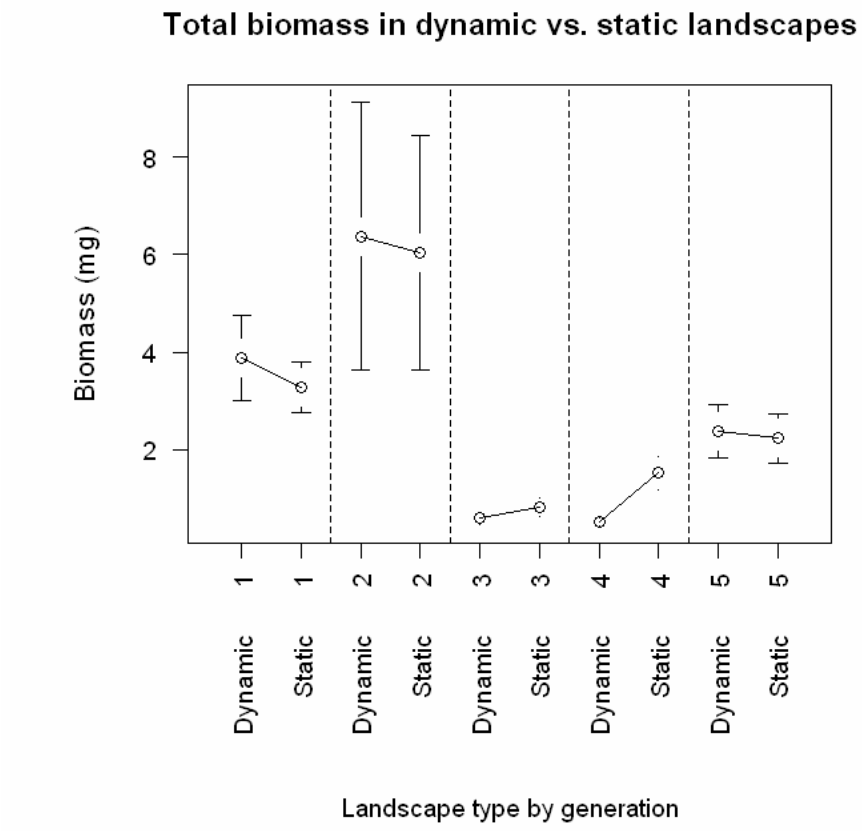


Figure 7 Total biomass in static vs. dynamic landscapes in generations 1–5. The mean and the 95% confidence interval are shown.

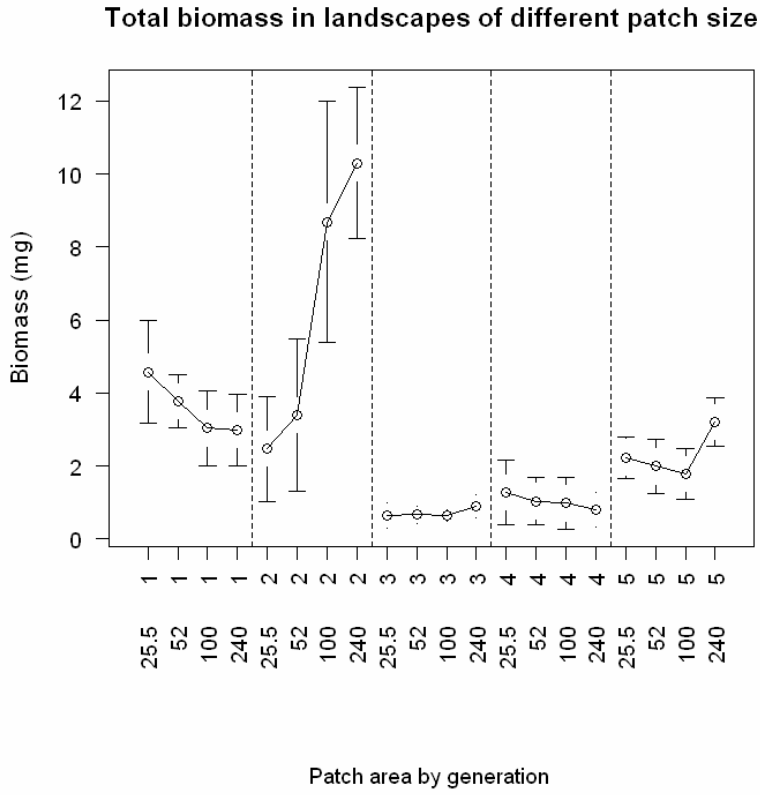
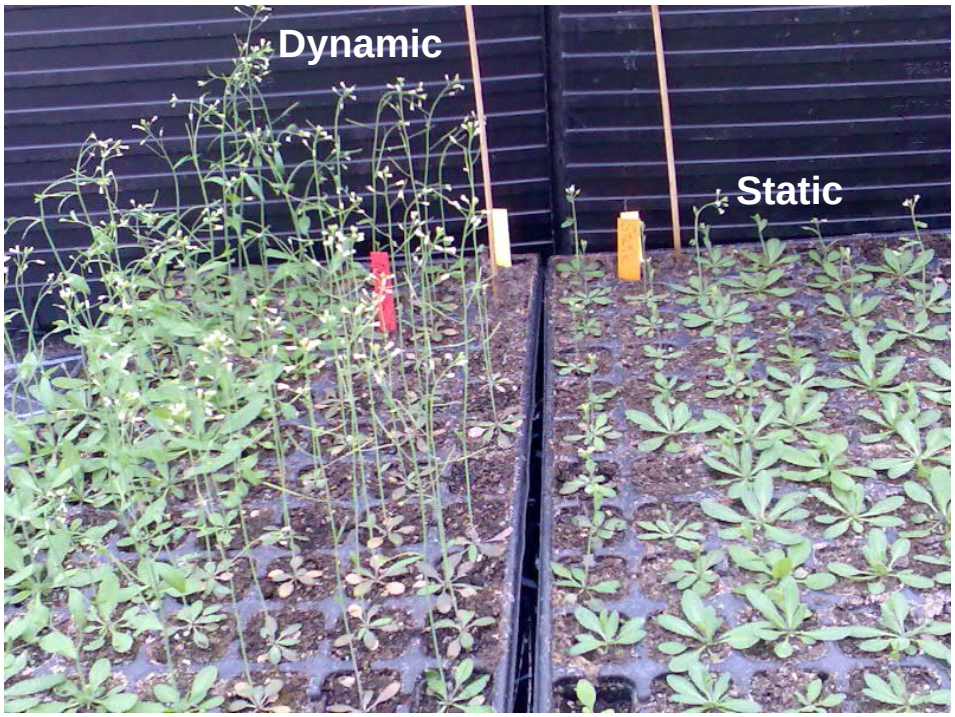


Figure 8 Total biomass in landscape of different patch area in generations 1–5. The mean and the 95% confidence interval are shown.

Chapter 5

A multi-generational landscape experiment with *Arabidopsis thaliana*. 2: Plant traits

Sima Fakheran, Cloé Paul-Victor and Lindsay A. Turnbull



ABSTRACT

We employed experimental metapopulations to investigate how landscape characteristics may influence plant morphological traits associated with dispersal ability in fragmented landscapes. We selected a population of 19 recombinant inbred lines (RILs) of *Arabidopsis thaliana*, varying in their seed mass and height. Ten of the selected lines carry the ERECTA mutation: these lines are expected to achieve poorer spatial dispersal of their seeds. We manipulated both the degree of fragmentation (by using four patch sizes), and the rate of patch disturbance, by using two different patch disturbance regimes (static vs. dynamic). Dynamic landscapes are characterized by continual patch destruction and regeneration, while patches in static landscapes remain in place for several generations. We measured the effects of five generations of selection on plant traits (height, number of branches and seed mass) in the 24 manipulated landscapes. To exclude the confounding effects of density and to confirm whether genuine selection had occurred, seeds sampled from generation 5 plants were grown under standardized conditions (a single plant per pot). We found a strong effect of disturbance regime on average plant height. There was strong selection for taller plants in dynamic compared with static landscapes. This resulted in both changes in the frequency of the ERECTA mutation and changes in the height of the surviving individuals, both ERECTA and non-ERECTA in static vs. dynamic landscapes. The absolute height in static landscapes was similar to the average height among the original lines while plants in dynamic landscapes were much taller. Plants in dynamic landscapes also had smaller seeds after 5 generations of selection, while the average seed mass in static landscapes was similar to the original lines. Thus, in the static landscapes there appears to have been very little selection on measured traits while in dynamic landscapes there was rapid evolution toward higher dispersal ability.

INTRODUCTION

In many plant species, populations are patchily distributed, such that areas of suitable habitat are scattered within an inhospitable matrix; i.e. metapopulations (Hanski 1983; Groom & Schumaker 1993; Hanski 1999; Cook *et al.*, 2002). However, such metapopulations span a whole range of spatial scales. In some species, each population is largely isolated from the others with relatively little dispersal between patches (although seed dispersal may still be the main vector of gene flow among fragments; Bacles et al., 2006). At the other extreme, patches may only contain a few individuals and the patches themselves may only be short-lived. If suitable patches remain in the same place year after year then limited dispersal is likely to be favoured, as most dispersing seeds will be lost to the matrix (Hastings 1983; Cheptou 2008). However, if suitable patches move around then good dispersal will be selected for, as species must track the environment. For example, annual plants often rely on small gaps free from competition with perennials, which are frequently created by disturbances of the perennial matrix (Carey & Watkinson 1993; Turnbull et al., 2007). For these annual plants, demography is influenced both by patch dynamics, and the colonization and extinction processes (Grubb 1986).

Colonization is a process which begins with dispersal (Richard 2000). Hence, in a fragmented landscape, connectivity between populations not only depends on the landscape dynamics and configuration, but also on the organism's dispersal ability (Gardner et al., 1987; Kindlmann et al., 2005; O'Neill et al., 1988; With & Crist 1995). For example, many sand-dune annual species, which rely on gaps in the perennial matrix, are short (< 10 cm tall) and lack specialised dispersal mechanisms; hence, dispersal is likely to be a passive process (Turnbull et al., 2004). In this case, characteristics of plant architecture are likely to have a strong influence on seed dispersion patterns (e.g. Nothon et al., 2003; Wender et al., 2005). For example, plant height and branching pattern as well as seed size (Thiede & Augspurger 1996; Wender et al., 2005).

We can see the importance of plant height by approximating the dispersal kernel (a statistical distribution of the seed dispersal pattern: Clark et al., 1999; Law et al., 2003) by an exponential distribution; a reasonable approximation for annual plants dispersing seeds in still air (Molofsky & Ferdy 2005). Increasing height inevitably increases the time taken for a seed to fall to the ground. Therefore, if a seed receives a small initial horizontal velocity, e.g. from slightly shaking the plant, it will inevitably travel further from the parent if released from a higher point (Soons & Bullock 2008). The equation for the exponential distribution is:

$$P(x) = \lambda \exp(-\lambda x) \qquad \text{eqn 1}$$

where λ is the rate parameter. The mean of the distribution is given by 1/ λ. Thus, halving the rate parameter doubles the mean dispersal distance, $\bar{d}$. Figure 1 reveals that doubling the average dispersal distance has a disproportionate effect on the tails of the distribution. Thus, a tall plant with a mean dispersal distance of 4 cm distributes 63% of its seeds within 4 cm of the parent, whereas a shorter plant (mean dispersal distance only 2 cm) distributes 86% of its seeds within 4 cm of the parent. Thus if these seeds compete for sites within 4 cm then the shorter plant has roughly 1.3 seeds to every seed of the taller plant. However, when considering long-distance dispersal, the tall plant ($\bar{d} = 4$) distributes 13% of its seeds more than 8 cm away from the parent, whereas the shorter plant ($\bar{d} = 2$) distributes 1.8% of its seeds more than 8 cm away from the parent. Thus, if these seeds compete for sites then the taller plant has roughly seven seeds to every one seed of the shorter plant. Thus we expect much strong selection against short genotypes in disturbed landscapes than against tall genotypes in undisturbed landscapes. The influence of the tail of the dispersal kernel (thin-tailed vs. fat-tailed), therefore has an important role on the pattern of colonization (Clark et al., 2001) .

Small seed mass is also often associated with enhanced dispersal (and hence disturbance) because, if a seed size/number trade-off operates, small-seeded plants will

produce more seeds (Smith & Fretwell 1974; Venable 1992; Turnbull et al., 1999; Nathan et al., 2002; Wender et al., 2005). In addition, under a seed size/number trade-off, disturbance increases the minimum viable seed size, because additional seeds are lost to disturbance events (Roy et al., 2004). Disturbance may also favour smaller seeds because they probably disperse passively over longer distances than larger seeds; for example, they are more easily carried along by light air movements (Finlay 2002, Jenkins et al., 2007). However; despite the advantages of small seeds, many plants choose to produce large ones (Tunbull et al., 1999). Plants may choose to produce large seeds because seedlings from large seeds generally survive better (Rees 1995; Tunbull et al., 1999) and seedlings from large seeds may have greater competitive ability (Rees & Westoby 1997; Tunbull et al., 1999). In static landscapes where densities are likely to build up (see chapter 4) then large seeds may have more of an advantage because competition is more intense.

Our experiment

Here we present an experimental study in which we manipulate landscape characteristics for populations of the annual plant *Arabidopsis thaliana.* We created artificial landscapes with islands of suitable habitat embedded in an unsuitable matrix to simulate islands of natural habitats in nature (Hanski 1999; Cook et al., 2002). We manipulated both the degree of fragmentation and the rate of patch disturbance by using two different patch disturbance regimes (static vs. dynamic). In static landscapes, suitable habitats remain suitable over several generations; but in disturbed or dynamic landscapes suitable habitat patches are only available for one generation. They are then destroyed and regenerated in new locations in the landscape (For more details see *Methods, Dynamic vs. static Landscapes*). We seeded the landscapes with nineteen recombinant inbred lines (RILs) of *Arabidopsis thaliana* differing dramatically in traits thought to be associated with dispersal and competitive ability; i.e. height and seed size. We then measured the effects of five generations of selection on these same plant traits.

In this chapter, the following questions are specifically addressed*:*

1) We hypothesise that long-range dispersal will be selected in dynamic landscapes and short-range dispersal in static landscapes. Thus, tall plants should be more successful in dynamic landscapes and shorter plants more successful in static landscapes.

2) Because of the overwhelming effect of the ERECTA mutation on plant height, we expect a higher frequency of the ERECTA mutation in static than in dynamic landscapes after 5 generations of selection. However, as the ERECTA mutation has sometimes been shown to reduce growth rates (Mitchell-Olds 1996) it might be eliminated from all landscapes.

3) If small seeds disperse further, we expect to find smaller-seeded lines in dynamic landscapes and if large seeds confer a competitive advantage under high-density conditions, we expect to find larger-seeded lines in static landscapes.

MATERIAL AND METHODS

Biological Material

We selected a population of 162 recombinant inbred lines (RILs) of *Arabidopsis thaliana* (Alonso-Blanco et al., 1999). The RILs are derived from reciprocal crosses between the two pure lines Landsberg *ERECTA* (L*er*), obtained as a mutant (*er*) from an accession of northern Europe (Rédei 1962; Rédei 1992), and Cvi, an accession from the tropical Cape Verde Islands (Lobin 1983). The two parents L*er* and Cvi have, respectively, small and large seeds (L*er*: 1.93 mg ± 0.10; Cvi: 3.51 mg ± 0.08; mass per 100 seeds, mean ± 1 SD; Alonso-Blanco 1999). The range in the seed mass exhibited by the entire RIL population (1.45-3.73 mg per 100 seeds) is greater than the variation expressed by the two parents.

Lines carrying the *ERECTA* mutation typically have short and upright stems, round leaves, short petioles and pedicels, flowers clustered at the top of the inflorescence, short and wide siliques with blunt tips, a compact inflorescence and reduced height (TAIR). The reduced height is the most striking thing about plants carrying the *ERECTA* mutation (Fig. 1).

Thus plants carrying this mutation are expected to achieve poorer spatial dispersal of their seeds (see *Introduction*). The presence of the *ERECTA* mutation has also been shown to reduce growth rates over a 15-day period (Mitchell-Olds 1996), although its presence does not affect final seed outputs (Paul-Victor et al., unpublished Ph.D. thesis).

We selected 19 lines from the possible 162. Originally, 30 lines plus the two parents were selected to use in experiments investigating the seed size/number trade-off (Paul-Victor et al., unpublished Ph.D. thesis). From these 30, we selected 17 plus the two parents to use in the landscape experiment described here. We selected these lines in such a way as to maintain the seed mass variation present in the original RIL population. The lines can inherit the mutation *ERECTA* from the L*er* parent. Ten of the selected lines carry this mutation, the other nine not (see Chapter 4: *Table. 1*). All seeds were obtained from The Arabidopsis Information Resource (TAIR).

One hundred seeds of each line were collectively weighed to give a sown seed mass estimate for each line. *ERECTA* and non- *ERECTA* lines did not differ significantly in their sown seed mass ($F_{1,17} = 0.091$, $p = 0.767$) and their ranges were similar (Fig. 2). Mean height values for each line are taken from Alonso-Blanco (1999), and represent the mean value from four individuals, grown in isolated pots. *ERECTA* and non- *ERECTA* lines differed dramatically in their height ($F_{1,17} = 51.07$, $p < 0.001$; Fig. 2). There was a negative correlation between height and seed size within each group, although it was not significant (Data from Alonso-Blanco (1999): Fig. 3; *ERECTA* lines, $\rho = -0.577$, df = 8, $p = 0.08$; non- *ERECTA* lines $\rho = -0.6$, df = 7, $p = 0.08$). These correlations must reflect genetic correlations within the RIL population. This can occur either when there is genetic linkage between seed size genes and genes controlling other traits, such as height, or when genes have pleiotropic effects (Williams 1957). Among the lines there is a trade-off between seed size and seed number, such that under certain conditions, lines producing small seeds produce more (Paul-Victor et

al., unpublished Ph.D. thesis), however the total mass of seeds produced is not related to seed size (Paul-Victor et al., unpublished Ph.D. thesis).

Landscapes

We set up a habitat fragmentation experiment using 24 landscapes with different degrees of fragmentation of the suitable habitat. The experiment was run for five generations in a glasshouse. Each landscape measured 90 × 64 cm, and consisted of patches in which *Arabidopsis* plants were allowed to grow (suitable habitat) and the matrix in which any plants growing were regularly removed (unsuitable habitat). The suitable habitat made up around 7 % of the total landscape. The suitable habitat consisted of 2, 4, 8 or 16 patches. The four patch sizes provide different degrees of habitat fragmentation and patch sizes were chosen to keep the total area of suitable habitat roughly constant. However, due to constraint of available material, the total area of suitable habitat varies slightly with the number of patches (see Chapter 4, *Table. 2*). There were six replicates of each level of habitat fragmentation making 24 landscapes in total (For more details on landscape construction see Chapter 4, *Landscapes*). At the end of generation 5, seeds were collected from a sample of individuals in each landscape and plants were grown in individual pots in a common garden experiment (see below: *Standardized Conditions*).

Dynamic vs. static Landscapes

As well as the patch size treatments, we imposed two different patch disturbance regimes. In the first (which we call static) seeds which fall into the natal patch are returned to the surface of a new patch in the next generation. To do this, seeds were manually released from the siliques (by gently shaking the plants by hand) once the plants were mature and seeds were ripe. All plant material was then measured and weighed (see *Data collection*). The surface layer of soil containing seeds was then scraped away from each patch and placed in a Petri

dish (one per patch) and placed in a fridge for one week. During this time all remaining soil was removed from the existing patches and replaced with fresh soil made up in the same way as before (50% soil and 50% sand). The new patches were then relocated at random within the landscapes and the seeds returned to the soil surface. Thus seeds which do not disperse away from their natal patch have a much higher chance of entering the next generation, although seeds which land in another patch, and not in the matrix, can also enter the next generation.

In the second patch disturbance regime (which we call dynamic) new Petri dishes (of the same number and size as the existing patches) were randomly placed around the landscape to collect dispersing seeds before plants began to flower (See Chapter 4). Seeds were then manually released from siliques in exactly the same way as for static landscapes. The new Petri dishes containing any dispersed seeds were removed and placed in the fridge for one week. All plants were removed and weighed and all patches randomly relocated and refilled with fresh soil. Thus in the dynamic landscapes, seeds falling back into the natal patch have no chance of entering the next generation. Seeds from static and dynamic landscapes were removed at the same time and placed in the same fridge together for the same length of time. Notice that, in static landscapes each patch maintains its identity through time (seeds taken from patch *i* are returned to the same patch *i*, but in dynamic landscapes a patch in generation $t+1$ cannot be identified with any particular patch in generation t.

Initialising landscapes

In generation 1, landscapes were initiated by introducing seeds of each of the 19 lines in the following way. The 19 selected lines were counted and sown so as to obtain equal initial densities in landscapes containing different patch sizes. We sowed one seed per line into each of the smallest patches, and 2, 4 and 8 seeds per line into each patch in the 8, 4 and 2-patch landscapes, respectively. Thus initially 16 seeds of each line were introduced into each

landscape. In total 7296 seeds were sown in the first generation in 180 patches of our 24 landscapes (See also *Timetable* in Chapter 4).

Data collection

The following variables used in the analyses presented here were measured at the same time point in each generation.

4) *Average seed mass*. Three combined samples were weighed from each landscape in week 8 of generations 2-5. Each combined sample consisted of 16 seeds, each sampled from a different individual. In 16-patch landscapes, a single individual from each patch was chosen; in 8-patch landscapes, 2 individuals from each patch were chosen, and so on.
5) *Final number of surviving adults* (week 9).
6) *Height and number of branches of 50% of the surviving adults* (week 9).

Standardized conditions

At the end of generation 5, 77 seed pods from 77 different plants in each landscape were sampled, labelled and kept separately in a cold room at 4 °C for one week. These seeds were then grown under standardized conditions (one plant per pot) to exclude the confounding effects of density and to measure the effects of 5 generations of selection on plant traits. Plants were grown in multi-pot trays of 77 pots/ tray (see Fig. 4). The 19 original lines (17 RILs + 2 parent lines) were grown again in these standardized conditions (4 replicates for each line). Pots were filled with the same 50% soil / 50% sand mixture, used in the experiment. Around 5 seeds from a single seed pod were sown into a pot and then thinned as soon as seedlings emerged to leave one plant per pot. Thirty-seven days after sowing we recorded the flowering stage of all surviving individuals (data not presented here). Forty-three days after sowing, the height and presence of the *ERECTA* mutation was recorded for all

surviving individuals. As the *ERECTA* mutation was difficult for a non-expert to identify, the identification was made by Masaki Kobayashi and Mathias Helling from Evolutionary Functional Genomics, Institute of Plant Biology, University of Zurich. Sixty-six days after sowing, 30 individuals from the original 77 in each landscape were randomly selected. The final height and number of branches of each individual were measured and we collected all non-dispersed seeds. We estimated the average seed mass of individuals within each landscape by weighing a single combined sample consisting of 150 seeds: 5 seeds from each of the 30 individuals (weighed on a microbalance).

Statistical analysis

Here we present the analysis of final height, number of branches and seed mass through generations 1–5 and for generation 5 individuals raised under standardised conditions. As part of the analysis of plant height, we analysed the frequency of the ERECTA mutation among surviving individuals. The ERECTA mutation is visible in the phenotype but could only be identified under standardised conditions. As seed mass was estimated from samples consisting of different numbers of seeds, we always standardised seed mass measurements to the mass of 100 seeds in milligrams (this also facilitates comparison with the data of Alonso-Blanco, 1999). Analyses were carried out using linear mixed effect models, using the function *lme* in the stats package R (Pinheiro & Bates 2000; R Development Core Team 2007). Landscape identity (i.e. 1–24) was treated as a random effect and formed the error term for the landscape-level treatments: patch size and patch disturbance treatments (static vs. dynamic) and their interactions. The frequency of the ERECTA mutation under standardised conditions was analysed using a glm assuming a binomial error distribution.

RESULTS

Original lines

The 19 original lines (17 RILs + 2 parent lines) were grown again under standardized conditions (4 replicate individuals per line). The final height, number of branches, and harvested seed mass (mass of 30 seeds) were recorded for each individual of the original lines, and correlations carried out on the line means. Harvested seed mass was considerably lower than sown seed mass (Fig. 6), indicating that the original lines produce smaller seeds when grown under standardized conditions. This could be due to our use of soil / sand mixture rather than 100% compost.

There was a negative correlation between height and sown seed mass in non-ERECTA lines (ρ = -0.856, df = 7, p = 0.003) and between height and harvested seed mass (ρ = -0.885, df = 7, p = 0.0015). For the *ERECTA* lines, this correlation between height and sown seed mass were also negative but not significant (Height vs. sown seed mass: ρ = -0.52, df = 8, p = 0.12; Height vs. harvested seed mass: ρ = -0.27, df = 8, p = 0.44). These correlations are very similar to those between seed mass and height found by Alonso-Blanco et al. (1999; see *Methods*). There was also a positive correlation between height and number of branches among ERECTA lines (ρ = 0.70, df = 8, p = 0.02), but not among non-ERECTA lines (ρ = 0.34, df =7, p = 0.37).

Height

Mean plant height through time

The effect of disturbance regime on mean plant height per landscape is shown in Fig. 5, and the effect of patch size in Fig.6. We fitted generation as a factor with five levels because of obvious non-linearity (Table. 1).

The effect of both disturbance regime and patch size vary with generation (generation × disturbance-regime, $F_{4,80}$ = 23.5, p < 0.0001; generation × patch area, $F_{4,80}$= 13.5, p <

0.0001). However, while the interaction disturbance regime × generation is significant, it is only the strength of the effect and not its direction that varies among generations. Thus, there was very little difference in height between static vs. dynamic landscapes in generations 1 and 2, but then the two populations diverged substantially with much taller plants in dynamic landscapes (Fig. 5). By generation 5, mean plant height in dynamic landscapes (19.8 cm ± 1.67) was more than twice the value in static landscapes (8.58 cm ± 1.14)**.** In contrast, for the patch size × generation interaction, the effect of patch size changes its strength and its direction among generations. There was a striking positive relationship between patch area and plant height in generation 2 only; however, there was only a weak relationship between patch area and height in the remaining generations (Fig. 6).

Plant height is of course highly plastic. Therefore, differences in plant height among treatments could also reflect differences in density. We therefore repeated the analysis including the final number of surviving adults per landscape as covariate (Table. 2). Density was log transformed because it was not expected to have a linear effect. Density had a significant negative effect on average plant height ($F_{1,79}$ = 111.708, $p < 0.0001$). Thus, as expected, higher density leads to shorter plants. However, after fitting this covariate, the main effect of disturbance regime and the interactions generation × disturbance-regime and generation × patch size remain significant (Table. 2). This suggests that some of the observed change in plant height is due to genuine selection and not just phenotypic plasticity.

Height under standardized conditions

There was a strong effect of disturbance regime on average height for plants grown under standardised conditions (Fig. 7). This difference could be due to a change in two things: 1) a change in the frequency of the ERECTA mutation between static and dynamic landscapes, and 2) changes in the height of surviving individuals of both types (ERECTA and non-ERECTA). To assess this, all plants were scored for the presence of the ERECTA mutation.

The frequency of the ERECTA mutation differed substantially between static and dynamic landscapes (Fig. 8, $\chi^2 = 295.48$, $p < 0.0001$). On average only 8 % (95% CI: 6.5 – 10.4) of individuals in dynamic landscapes carried the mutation, and in 3 of the 12 dynamic landscapes the ERECTA mutation was completely eliminated. In contrast, 44% (95% CI: 37 – 51) of individuals carried the ERECTA mutation after 5 generations in static landscapes, close to the original frequency (52.6 %). Thus, as expected the ERECTA mutation has a serious disadvantage in a landscape where good dispersal is necessary for survival.

There was also a significant patch area × disturbance-regime interaction on the frequency of the ERECTA mutation (Table. 5). This interaction was weak compared to the main effect, but nevertheless significant (χ^2 =16, $p < 0.0001$). The significant interaction occurred because the frequency of ERECTA increased with patch area in dynamic landscapes, but decreased with patch area in static landscapes. However, it seems that this effect is mainly due to the largest patch treatment in which the difference between static and dynamic landscapes is less pronounced (see Fig. 8).

As well as selection for or against the ERECTA mutation, differences in average height among landscapes can also be due to changes in the average height of the surviving individuals, both ERECTA and non-ERECTA. To assess this, we measured the final height of 30 randomly-chosen individuals from the 77 plants grown from individuals sampled from each landscape in generation 5 (Fig. 9). Because of the unbalanced nature of the data we could not fit a model containing the 3 terms: disturbance regime, patch area and the ERECTA mutation, plus their interactions. We therefore began by fitting a model with the experimental treatments disturbance regime and patch area plus their interaction. However, only the disturbance treatment was significant (Table. 3). We then, fitted a second model containing the terms ERECTA mutation and disturbance regime plus their interaction. This reveals that individuals carrying the ERECTA mutation are on average 6.23 cm (95% CI: 3.35 – 9.11) shorter in static landscapes while non- ERECTA individuals are 10.83 cm (95% CI: 8.4

—13.23) shorter in static landscapes (Table. 4). The overall average height difference in static vs. dynamic landscapes is in fact 15.39 cm (95% CI: 14.81 — 15.98), that is greater than the difference in height between either ERECTA or non-ERECTA individuals. The large overall difference in height between static and dynamic landscapes is therefore due to both changes in the frequency of ERECTA and selection on the height of surviving plants.

We also measured the height of four individuals from each of the original 19 lines grown under standardised conditions. The average height of the original lines is 21.9 cm (95% CI: 19 — 24.8 cm) while the mean height of plants in static landscapes is 20.1 cm (95% CI: 19.7 — 20.5 cm) and the mean height in dynamic landscapes is 35.5 cm (95% CI: 35.2 — 35.8 cm). This indicates strong directional selection on height in dynamic rather than in static landscapes.

Branches

The effect of disturbance regime on mean number of branches per landscape through generations 1-5 is shown in Fig. 10, and the effect of patch size in Fig. 11. The number of branches was much higher in generation 1 than in the other four generations, and because we included generation 1 in this analysis, we fitted generation as a factor with 5 levels. Generation was highly significant (Table. 6). The very high degree of branching in generation 1 probably occurred because densities were very low, as we only introduced a small number of seeds by hand (see Methods, *Initialising landscapes*). The main effect of disturbance regime is large ($F_{1,20}$ = 27.64, $p < 0.0001$) compared to the interaction (generation × disturbance-regime, $F_{4,80}$ = 2.87, p = 0.03), and reveals that plants in dynamic landscapes have more branches than those in static landscapes (Fig. 10). The generation × patch size interaction is also significant ($F_{4,80}$ = 7.59, $p < 0.001$) and in contrast to the patch size × generation interaction, the effect of patch size changes its strength and direction among generations.

Differences in branching pattern among treatments could also reflect differences in density. Therefore, we performed a second analysis with seedling density fitted first as covariate. This was highly significant (Table. 7, $F_{1,79} = 531.58$, $p < 0.001$) and its inclusion in the model made the main effect of patch disturbance regime completely non-significant (Table. 7). Hence the differences in the mean number of branches in static vs. dynamic landscapes seem to be entirely due to differences in density. This was confirmed when plants were grown under standardised conditions where there were no significant treatment effects (Table. 8, Fig. 12).

Seed mass

Seed mass also changed through the generations 2–5 (Fig. 13). However, there appeared to be a clear linear effect of generation which was therefore fitted as a continuous variable. There was a highly significant decline in seed mass in dynamic landscapes over time, but not in static landscapes (disturbance regime × generation interaction, $F_{1,256} = 45.94$, $p < 0.0001$). There was also a significant patch area × disturbance regime interaction (Table. 9), such that seed size declined with increasing patch area in dynamic landscapes and increased with patch area in static landscapes (Fig. 14). We performed a second analysis including the final number of surviving adults per landscape as covariate (Table. 10). Density was log-transformed because it was not expected to have a linear effect. Density had a significant positive effect on average seed mass per landscape (Table. 10). However, after fitting this covariate, the interactions generation × disturbance-regime and patch size × disturbance-regime remain significant (Table. 10).

To obtain a measure of the average seed mass per landscape under standardized conditions, we weighed a single sample of 150 seeds consisting of 5 seeds from each of 30 randomly-selected individuals. There was a strong effect of disturbance regime on seed mass (Table. 11, $F = 32.86$, $p < 0.0001$), such that after five generations of selection plants from

static landscapes had heavier seeds than those in dynamic landscapes (Fig. 15). There was no patch area effect on seed mass (Table. 11).

We also weighed a sample of 30 seeds from individuals of each of the 19 original lines grown under the same standardized condition. This was used to calculate an average or expected seed mass for a sample of 150 seeds, assuming all lines had equal representation. This value, 2.00 (CI: 1.78 — 2.22; standardised to the mass per 100 seeds), is closer to that observed in static landscapes, 1.95 (CI: 1.67 — 2.23) rather than that in dynamic landscapes, 1.48 (CI: 1.37 — 1.60) indicating that dynamic landscapes have diverged more strongly from the ancestral population.

DISCUSSION

In Chapter 4, we saw that the disturbance treatment, static vs. dynamic had large effects on seedlings density in generations 3—5, indicating local dispersal in generations 2—4. In the introduction to this chapter we also saw that increasing height is very likely to lead to increased long-distance dispersal, hence tall plants should be strongly favoured in dynamic landscapes. In this chapter we saw that there was indeed strong selection for tall plants in dynamic compared with static landscapes. This occurred in two ways: First, the frequency of the ERECTA mutation declined to around 8 % in dynamic landscapes compared with 44 % in static landscapes. Second, the height of the surviving individuals whether ERECTA or non-ERECTA was greater in dynamic compared with static landscapes. The overall height difference between the two landscape types by generation 5 was almost 15 cm; an impressive and visible difference (see picture on the first page of this chapter). The difficulty in effectively dispersing over such seemingly small distances confirms the results of Molofsky and & Ferdy (2005). They used an experimental metapopulation of the annual plant (*Cardamine pensylvanica*) and showed that extinction risk increased dramatically with increasing distance between local populations.

Interestingly, although there was strong selection for tall plants in dynamic landscapes, there appeared to be much weaker selection for short plants in static landscapes. Thus, the average height in static landscapes is similar to the average height among the original lines. This is despite the fact that tall plants in small patches inevitably lose a higher fraction of seeds to the matrix (see *Introduction*). There are two likely reasons for the asymmetry in selection on height. First, under an assumption that dispersal kernels are roughly exponential (see *Introduction*), an increase in height has a disproportionate effect on the tail of the distribution. Thus, most seeds fall close to the parents even for tall plants. Second, in static landscapes densities are higher and the height of all plants is reduced. This probably means that tall genotypes are also short in high-density static landscapes due to phenotypic plasticity. This contrasts with a study of natural populations of *Crepis sancta* in urban and more natural conditions (Cheptou 2008). *Crepis sancta* produces two types of seeds: dispersing and non-dispersing. In urban patches, most dispersing seeds are lost and hence individuals in urban environments have evolved to produce a lower fraction of dispersing seeds. Thus, here the costs of investment in long-distance dispersal are clearly penalized in a static landscape. This is similar to the finding on islands that many species stop investing in long-distance dispersal (Cody & Overton 1996).

Within our original lines there is the problem that height and seed mass are negatively correlated within groups (i.e. among both ERECTA and non- ERECTA lines). Thus, we cannot unequivocally assume that taller genotypes are better in dynamic landscapes, because selection could have primarily acted on seed size. This would also be plausible, as under certain conditions, small-seeded individuals produce more seeds (Smith & Fretwell 1974; Venable 1992; Turnbull et al., 1999; Nathan et al., 2002; Wender et al., 2005), and therefore have a greater chance of colonising new patches. However, the inclusion in our design of genotypes carrying the ERECTA mutation allows us to separate the effects of height and seed mass to some degree. Thus, if selection acted primarily on seed size in dynamic landscapes,

small-seeded genotypes carrying the ERECTA mutation should have been just as fit as small-seeded non-ERECTA genotypes. However, this seems not to have been the case, as lines carrying the ERECTA mutation were almost eliminated in dynamic landscapes. Thus, interestingly, this is one of the few examples where the shape of the dispersal kernel rather than seed production *per se* has experienced strong selection (Levine & Murrell 2003).

In static landscapes there appears to have been very little selection on measured traits. Particularly, there dose not seem to have been selection for large- seeded genotypes. This is despite the fact that competition was intense in static landscapes with a large number of seedlings failing to survive to adulthood (Chapter 4). There is therefore the intriguing possibility that seed size in static landscapes was selectively neutral (Turnbull et al., 2008). If bigger-seeded genotypes produce fewer seeds but these seeds survive better, then these two factors can theoretically cancel out. However, it is perhaps surprising that large seed size does not confer a size-asymmetric competitive advantage under such high density conditions (Weiner 1986; Weiner 1990; Weiner et al., 2001; Stoll et al., 2002). It is possibly the case that the range in seed mass present in the original lines is insufficient to confer asymmetric competitive differences. In communities of sand- dune annual species, to which *Arabidopsis thaliana* belongs, the range in seed size is typically larger (Rees 1995). Alternatively, our system was perhaps too nutrient-poor to experience asymmetric competition, which is generally associated with competition for light (Vojtech et al., 2007).

There is a large literature on the differential negative effects of both disturbance and competition, which are both considered to reduce diversity. This has lead to the proposal that an intermediate level of disturbance; which in itself has relatively little direct effect, but prevents competitive exclusion by superior species or genotypes, will maximise diversity (Connell 1978; Roxburgh et al., 2004). However, in our experiment, disturbance seems to have imposed a much stronger selection pressure than competition. Therefore, in dynamic landscapes characterised by patch destruction and regeneration, only individual plants with

sufficiently long-range dispersal can survive (see also Johst et al., 2002), and there seems to have been rapid elimination of many of the original genotypes. However, despite the higher levels of seedling death in static landscapes, there is evidence that a much greater variety of genotypes have survived. This is rather unexpected and perhaps indicates that habitat disturbance is a much more potent force for the removal of genetic variation than competition, at least for annual plants.

Most experimental and observational studies of habitat fragmentation have focused on discrete but stable habitats and conclude that fragmentation could lead to a decrease in dispersal rate and on positive selection for non-dispersing individuals, because genes of dispersing seeds will be lost from isolated populations (Cheptou 2008). Our study compared both stable and unstable fragmented habitats (static vs. dynamic landscapes) and demonstrated strong selection for good dispersal ability in habitats where the remaining fragments are disturbed. This confirms the results of Johst et al., (2002) who reached the same conclusion using a spatially explicit metapopulation model. In addition, in our landscape experiment, disturbance regime was more important than area of the habitat patches, both in landscape-level and individual plant traits. We conclude that when assessing the potential ecological impacts of habitat fragmentation the temporal variability of the habitat patches as well as the spatial variability of the patches needs to be taken into account.

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Table 1 ANOVA results of the analysis of mean plant height per landscape through generations 1–5. We analysed a single mean value per landscape based on measurements of 50% of surviving individuals.

	numDF	denDF	F-value	p-value
(Intercept)	1	80	24816.533	<.0001
Disturbance-regime	1	20	89.496	<.0001
Generation	4	80	72.778	<.0001
log(Patch.size)	1	20	8.987	0.0071
Disturbance-regime × Generation	4	80	23.507	<.0001
Disturbance-regime × log (patch.size)	1	20	0.466	0.5026
Generation × log(Patch.size)	4	80	13.479	<.0001
Disturbance-regime × Generation × log(Patch.size)	4	80	0.216	0.929

Table 2 ANOVA results of the analysis of mean plant height per landscape through generations 1–5. We analysed a single mean value per landscape based on measurements of 50% of surviving individuals, but fitting density as covariate.

	numDF	denDF	F-value	p-value
(Intercept)	1	79	24737.93	<.0001
log (density)	1	79	111.708	<.0001
Disturbance-regime	1	20	30.98	<.0001
Generation	4	79	63.573	<.0001
log(Patch.size)	1	20	11.603	0.0028
Disturbance-regime × Generation	4	79	18.388	<.0001
Disturbance-regime × log (patch.size)	1	20	0.488	0.4927
Generation × log(Patch.size)	4	79	13.6	<.0001
Disturbance-regime × Generation × log(Patch.size)	4	79	0.205	0.9351

Table 3 ANOVA results of the analysis of the final height of generation 5 plants grown under standardized conditions. The final heights of 30 individuals per landscape were measured.

	numDF	denDF	F-value	p-value
(Intercept)	1	696	3170.917	<.0001
Disturbance- regime	1	20	243.331	<.0001
log(Patch.size)	1	20	1.042	0.3195
Disturbance-regime × log(Patch.size)	1	20	0.793	0.3837

Table 4 ANOVA results of the analysis of the final height of generation 5 plants grown under standardized conditions, but with disturbance regime and presence of the ERECTA mutation as explanatory variables.

	numDF	denDF	F-value	p-value
(Intercept)	1	694	4547.562	<.0001
Disturbance-regime	1	22	348.972	<.0001
ERECTA	1	694	103.189	<.0001
Disturbance-regime × ERECTA	1	694	27.843	<.0001

Table 5 ANOVA results of the frequency of ERECTA in different treatments after 5 generations of selection. The frequency was assessed from generation 5 plants grown under standardized conditions. Frequencies are based on 77 plants per landscapes.

	Df	Deviance	Resid. Df	Resid. Dev	P(>|Chi|)
NULL			23	502.51	
log(Patch.size)	1	0.14	22	502.36	0.71
Disturbance-regime	1	295.48	21	206.88	3.18E-66
log(Patch.size) × Disturbance-regime	1	16	20	190.88	6.33E-05

Table 6 ANOVA results of mean number of branches per landscape through generations 1–5. We analysed a single mean value per landscape based on measurements of 50% of surviving individuals.

	numDF	denDF	F-value	p-value
(Intercept)	1	80	584.7673	<.0001
Disturbance-regime	1	20	27.6405	<.0001
Generation	4	80	81.4872	<.0001
log(Patch.size)	1	20	2.7918	0.1103
Disturbance-regime × Generation	4	80	2.8723	0.0281
Disturbance-regime × log(Patch.size)	1	20	0.1095	0.7442
Generation × log(Patch.size)	4	80	7.5902	<.0001
Disturbance-regime × Generation × log(Patch.size)	4	80	1.38	0.2483

Table 7 ANOVA results of mean number of branches per landscape through generations 1–5. We analysed a single mean value per landscape based on measurements of 50% of surviving individuals, but fitting density as covariate.

	numDF	denDF	F-value	p-value
(Intercept)	1	79	531.5865	<.0001
log(density)	1	79	232.2094	<.0001
Disturbance-regime	1	20	0.1852	0.6716
Generation	4	79	61.3036	<.0001
log(Patch.size)	1	20	4.0724	0.0572
Disturbance-regime × Generation	4	79	0.5198	0.7214
Disturbance-regime × log(Patch.size)	1	20	0.0041	0.9493
Generation × log(Patch.size)	4	79	11.9582	<.0001
Disturbance-regime × Generation × log(Patch.size)	4	79	1.8136	0.1345

Table 8 ANOVA results of the analysis of the final number of branches for generation 5 plants grown under standardized conditions. We counted the number of branches on each of 30 plants per landscape.

	numDF	denDF	F-value	p-value
(Intercept)	1	696	966.0036	<.0001
Disturbance-regime	1	20	0.0294	0.8655
log(Patch.size)	1	20	0.2354	0.6328
Disturbance-regime × log(Patch.size)	1	20	0.2397	0.6298

Table 9 ANOVA results of the analysis of mean seed mass per landscape through generations 2–5. Three combined samples of 16 seeds, each taken from a different individual, were weighed from each landscape in each generation.

	numDF	denDF	F-value	p-value
(Intercept)	1	256	2489.5428	<.0001
Disturbance-regime	1	20	3.3122	0.0838
Generation	1	256	19.6999	<.0001
log (Patch-size)	1	20	0.0775	0.7835
Disturbance-regime × Generation	1	256	42.2045	<.0001
Disturbance-regime × log(Patch-size)	1	20	8.5998	0.0082
Generation × log(Patch-size)	1	256	0.4807	0.4887
Disturbance-regime × Generation × log(Patch-size)	1	256	3.7886	0.0527

Table 10 ANOVA results of the analysis of mean seed mass per landscape through generations 2–5. Three combined samples of 16 seeds, each taken from a different individual, were weighed from each landscape in each generation, but fitting density as covariate.

	numDF	denDF	F-value	p-value
(Intercept)	1	255	2436.0968	<.0001
log(density)	1	255	10.9067	0.0011
Disturbance-regime	1	20	0.4172	0.5257
Generation	1	255	26.292	<.0001
log (Patch-size)	1	20	0.0064	0.9368
Disturbance-regime × Generation	1	255	27.9478	<.0001
Disturbance-regime × log(Patch-size)	1	20	8.5982	0.0082
Generation × log(Patch-size)	1	255	0.4042	0.5255
Disturbance-regime × Generation × log(Patch-size)	1	255	3.6792	0.0562

Table 11 ANOVA results of the analysis of seed mass per landscape of generation 5 plants grown under standardized conditions. A single combined sample of 150 seeds, consists of 5 seeds from each of 150 different individuals from each landscape, was measured.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Disturbance-regime	1	2.9456	2.9456	32.8642	1.31E-05
log(Patch.size)	1	0.00895	0.00895	0.0999	0.7552
Disturbance-regime × log(Patch.size)	1	0.06701	0.06701	0.7476	0.3975
Residuals	20	1.79259	0.08963		

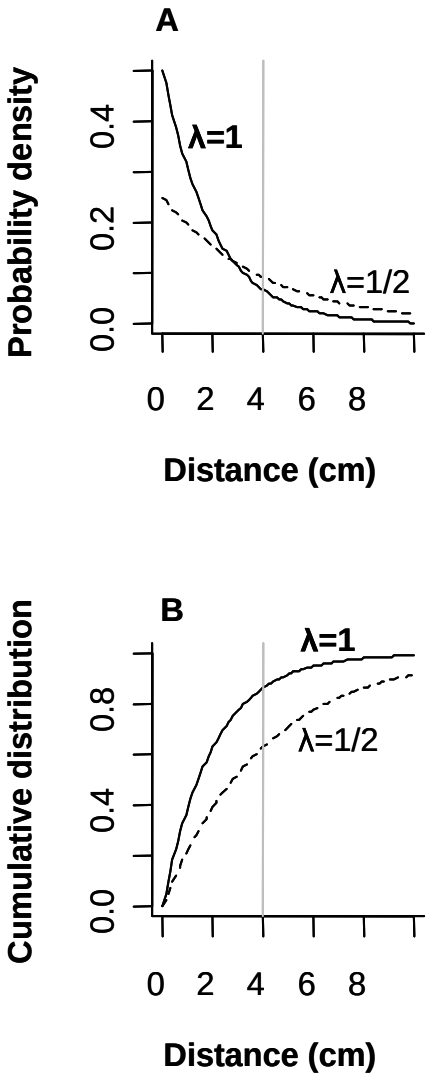

Figure 1 The effect of doubling the mean dispersal distance (1/ λ), on the probability density and the cumulative probability distribution, assuming dispersal can be described by an exponential distribution. A) The effect of doubling the dispersal distance on the probability density function, i.e. the probability that a seed travels a given distance away from the parent. B) The same, but showing the cumulative distribution functions.

Figure 2 ERECTA (left) vs. non-ERECTA (right); the reduced height is the most striking thing about plants carrying the ERECTA mutation.

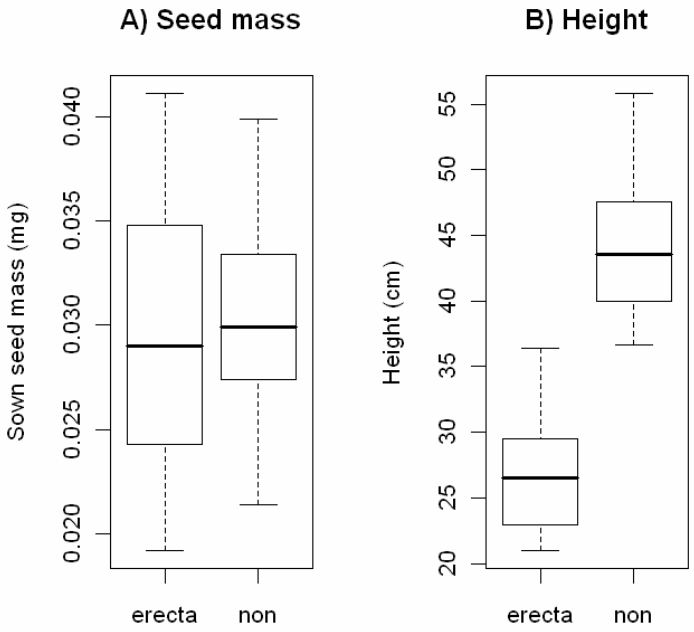

Figure 3 *ERECTA* and non-*ERECTA* lines have similar seed mass distributions, but they differ dramatically in their heights (see Fig. 1). Bold horizontal lines show the median, boxes show the interquartile range, and the whiskers show the maximum and minimum values.

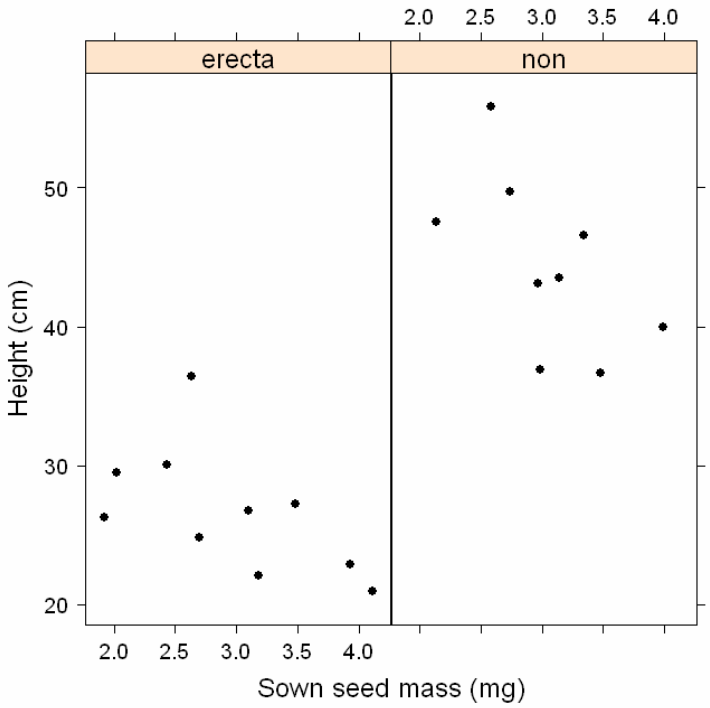

Figure 4 The relationship between sown seed mass (mean of 100 seeds) and height (cm) recorded in Alonso-Blanco et al. (1999), for the 19 lines used in the experiment described here.

Figure 5 Fifth-generation plants grown under standardized conditions (a single plant per pot). Plants were reared from seeds sampled in the fifth generation and were grown alone in multi-pot trays of 77 pots/ tray to exclude density effects. The 19 original lines were also grown under the same standardized conditions (4 individuals per line; Right).

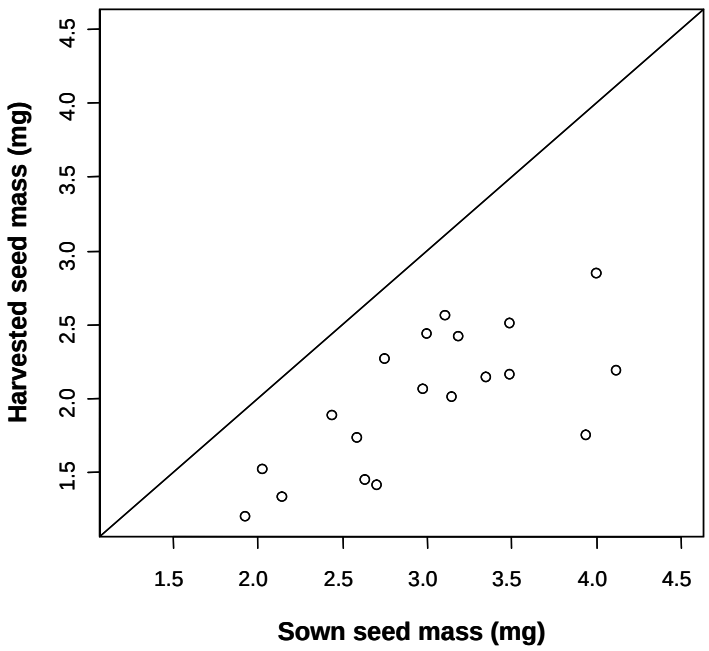

Figure 6 Sown seed mass vs. harvested seed mass for the 19 original lines when grown under standardized conditions. Each point represents the mean of four individuals.

The original lines produced smaller seeds when grown under standardized conditions, i.e. harvested seed mass was consistently lower than sown seed mass. The 1:1 is also shown.

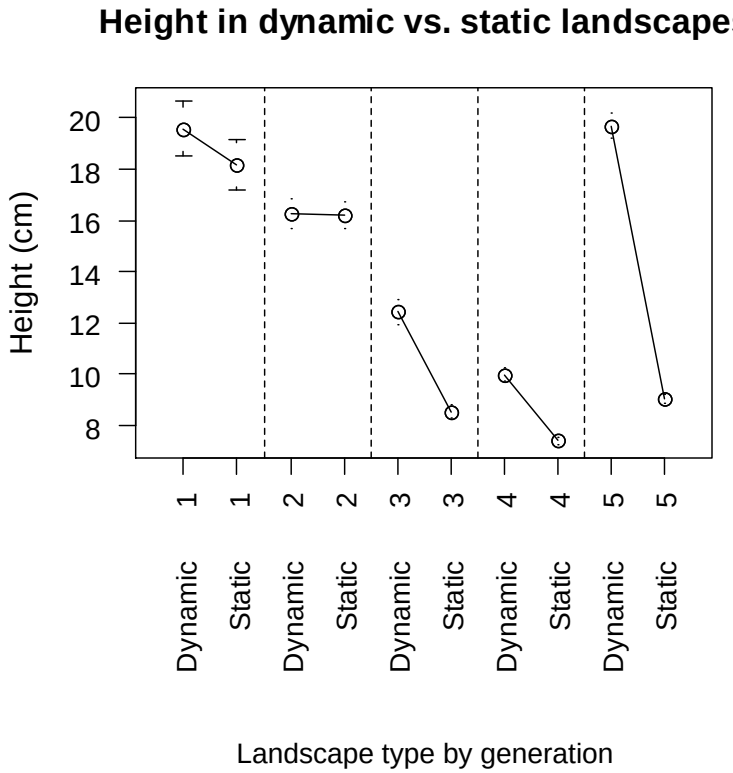

Figure 7 Average plant height (cm) in static vs. dynamic landscapes in generations 1–5. The mean and 95% confidence interval are shown.

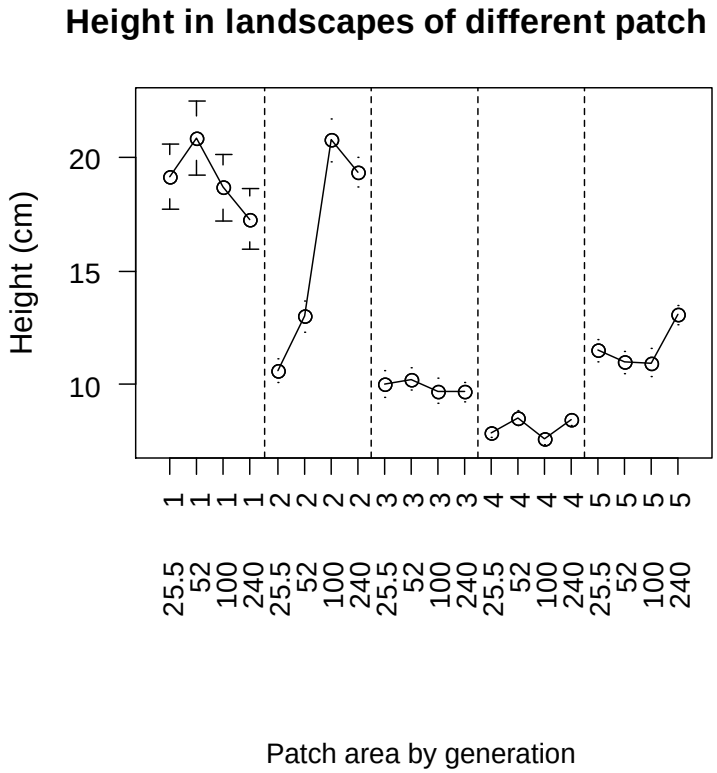

Figure 8 Average plant height in landscapes of different patch area in generations 1–5. The mean and 95% confidence interval are shown.

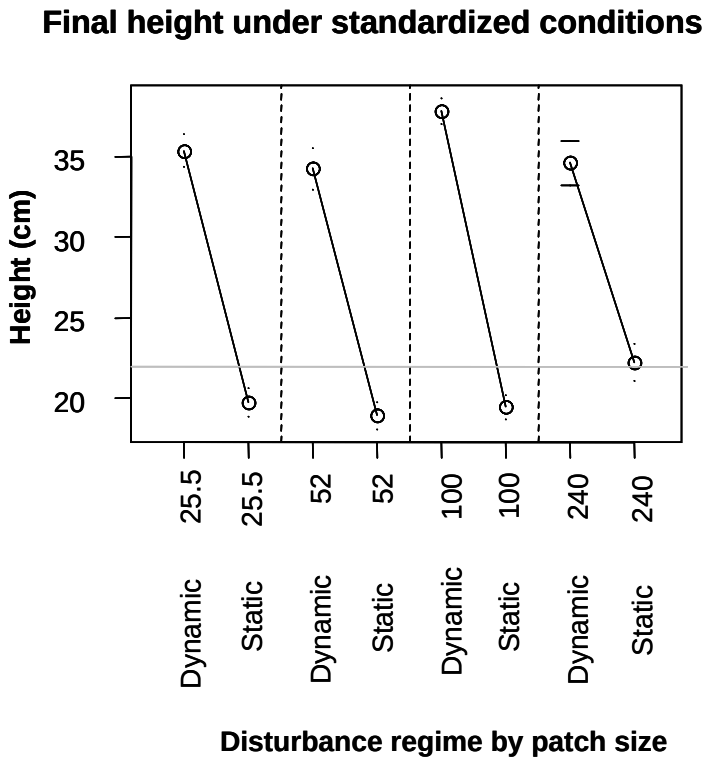

Figure 9 The final height of individuals from generation 5 grown under standardized conditions. The mean and 95% confidence interval for each disturbance regime and patch area condition are shown. The mean height of the original lines grown under the same standardized conditions is shown by the grey line.

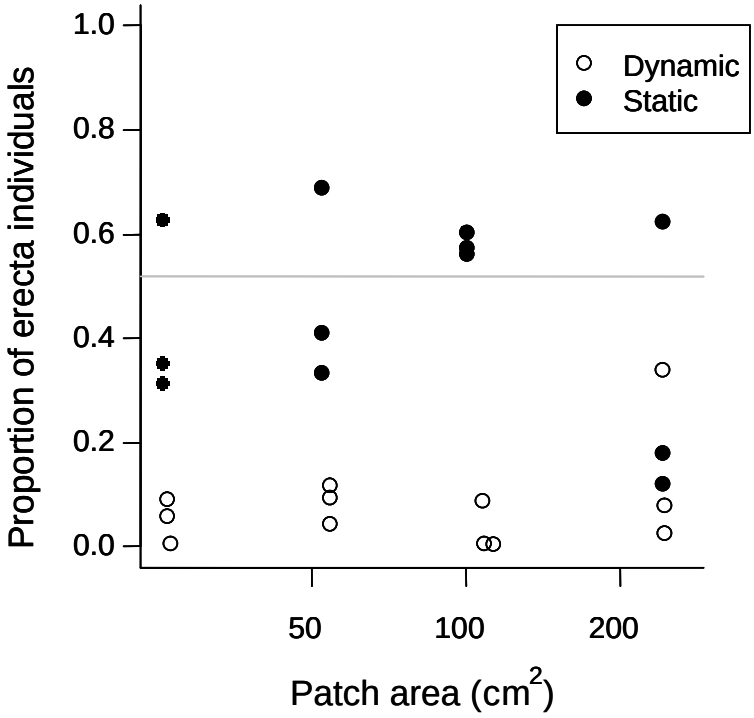

Figure 10 The frequency of the ERECTA mutation in each of 24 landscapes (dynamic vs. static) of different patch area. The frequency of the ERECTA mutation among the original lines is shown by the gray line.

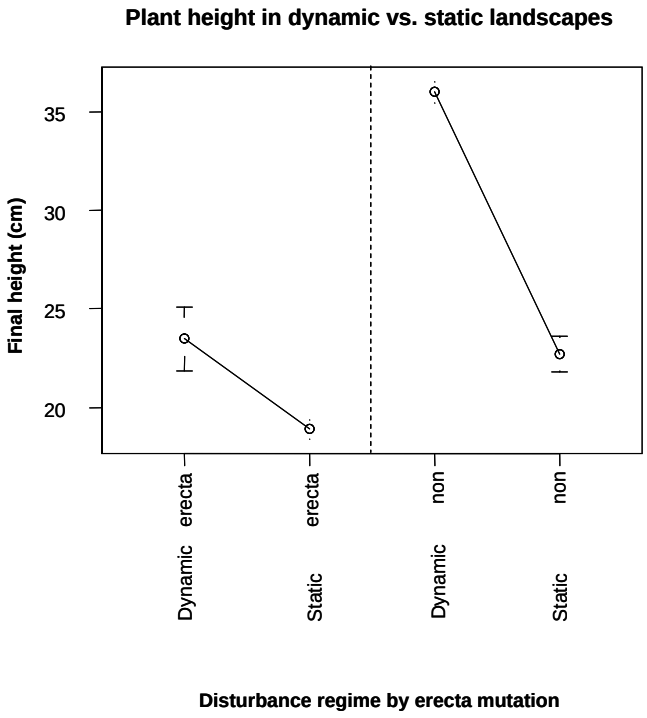

Figure 11 The height of generation-5 individuals from static vs. dynamic landscapes grown under standardized conditions. Individuals are grouped according to whether or not they carry the ERECTA mutation. The mean and 95% confidence interval are shown.

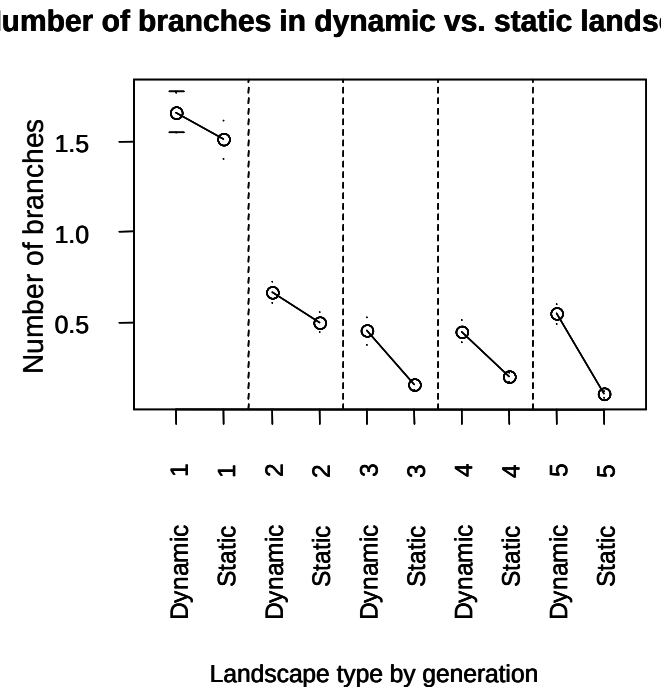

Figure 12 The average number of branches in static vs. dynamic landscapes in generations 1–5. The mean and 95% confidence interval are shown.

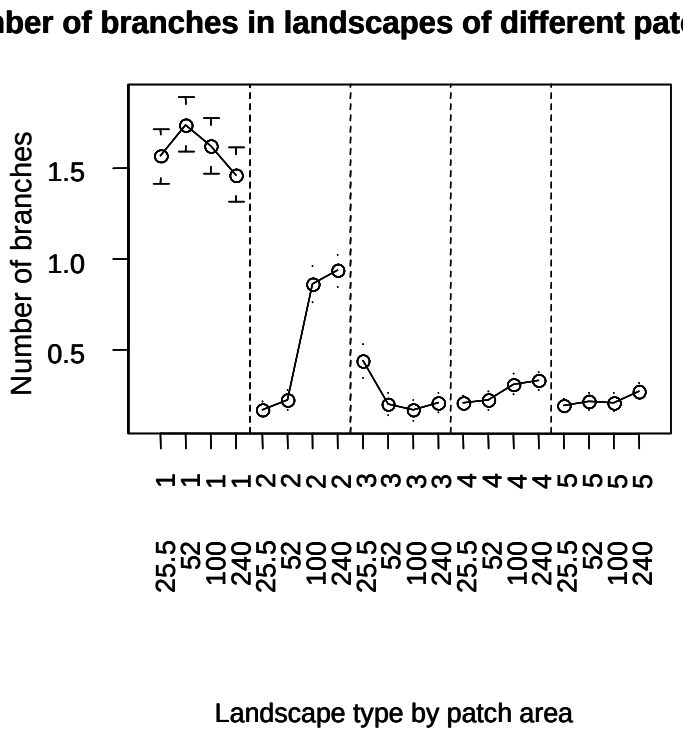

Figure 13 The average number of branches in landscapes of different patch area in generations 1–5. The mean and 95% confidence interval are shown.

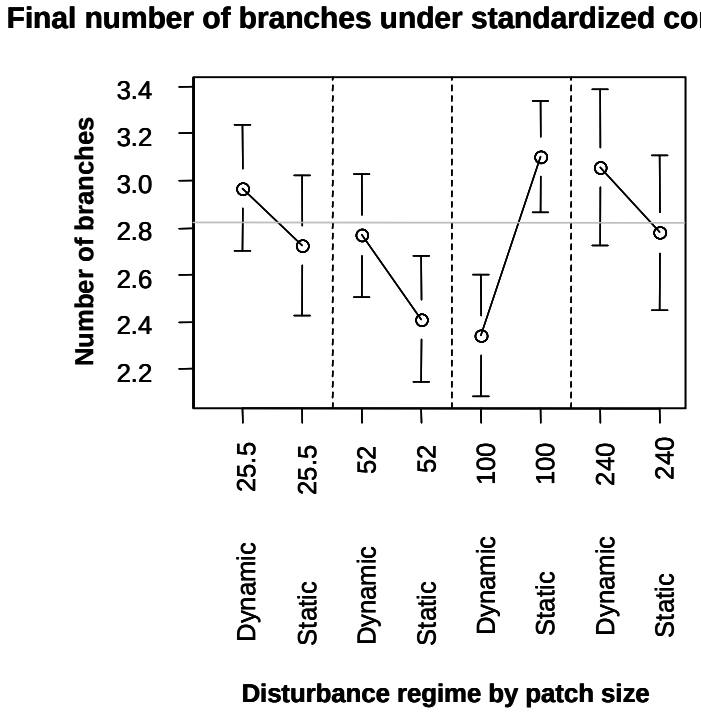

Figure 14 The final number of branches from generation-5 individuals grown under standardized conditions. The mean and 95% confidence interval for each disturbance regime and patch area condition are shown. The mean number of branches of the original lines grown under the same standardized conditions is shown by the grey line.

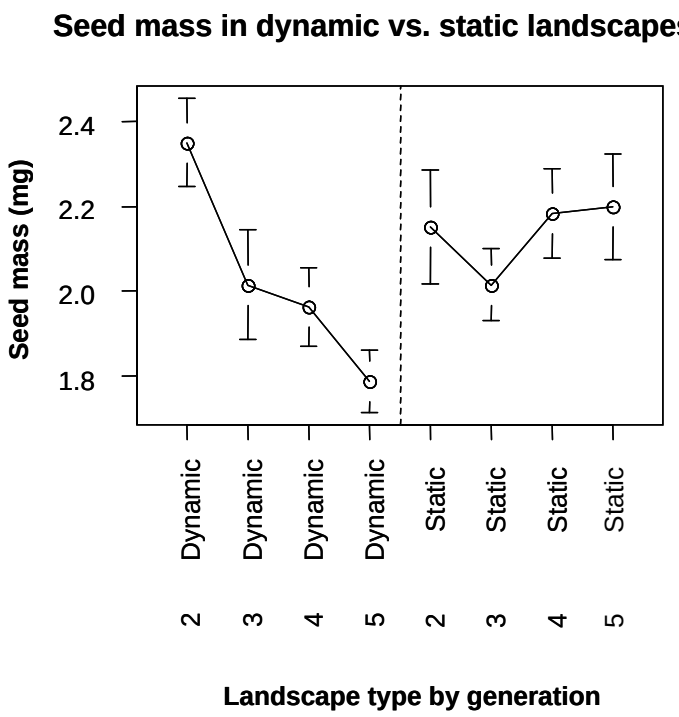

Figure 15 The average seed mass in landscapes exposed to different disturbance regimes (static vs. dynamic) through generations 2–5. These are then standardised to the mass of 100 seeds to facilitate comparisons. The mean and 95% confidence interval are shown.

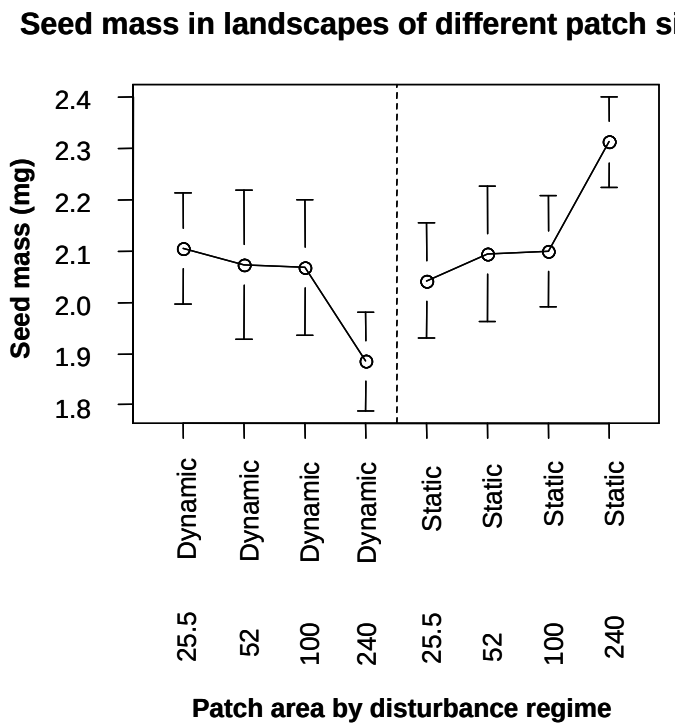

Figure 16 The average seed mass in landscapes exposed to different disturbance regime (static vs. dynamic) and patch area combinations. These are then standardised to the mass of 100 seeds to facilitate comparisons. The mean and 95% confidence interval are shown.

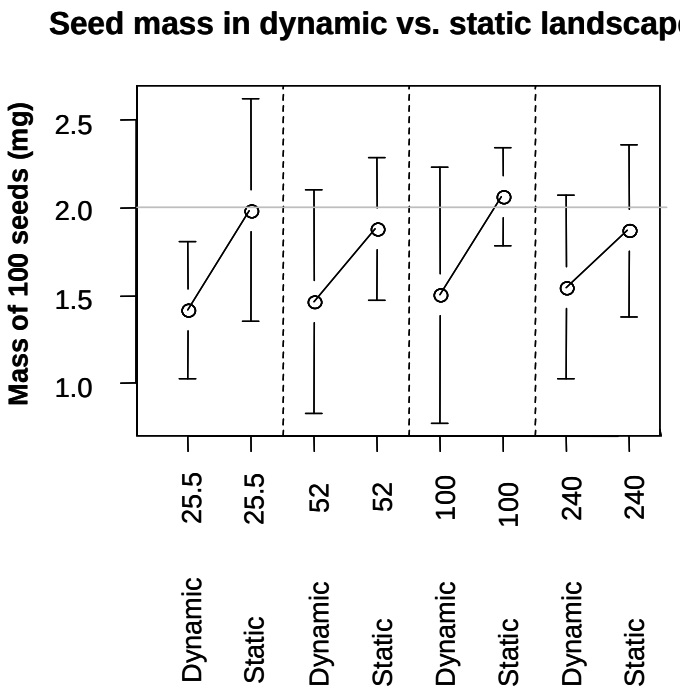

Figure 17 Mean seed mass and disturbance regime (static vs. dynamic) in landscapes with different patch size for generation-5 plants grown under standardized conditions. Mean seed mass was measured as a single combined sample of 150 seeds, consisting of 5 seeds from each of 30 individuals. These are then standardised to the mass of 100 seeds to facilitate comparisons. The mean and 95% confidence interval are shown. The average or expected mass of 100 seeds of the original lines are equally represented in the sample is shown by the grey line. This is calculated from the mean seed mass produced by each line grown under the same standardized conditions.

Chapter 6

General Discussion

GENERAL DISCUSSION

In my Ph.D. thesis, I conducted both observational and experimental studies on the effects of landscape patterns (habitat fragmentation and spatial structure of landscape elements) and landscape dynamics (changes in landscape patterns through time) on plant communities. I investigated evolutionary and ecological processes in spatially structured landscapes at different scales from natural landscapes to experimental microcosms. This involved the monitoring of large scale population processes in rather temporally stable fen meadows, as well as experimentally manipulated short-lived metapopulations in temporally unstable habitat patches.

To date, many researchers have focused on the effects of habitat fragmentation, habitat isolation, inter patch distance and landscape connectivity on a single or few species (e.g. MacArthur & Wilson 1967; Taylor et al., 1993; Fahrig & Merriam 1994; Hanski 1999). However, there is a lack of studies on the relationship between landscape patterns and biodiversity (Hersperger 2006). My PhD thesis helped to fill this gap by increasing our understanding of the response of whole plant communities, i.e. their composition and biodiversity, to landscape patterns.

In addition, a common approach in habitat fragmentation studies is to survey habitat fragments for presence or absence of focal species at a particular point in time, i.e. snapshots (Gu et al., 2002), without considering that all landscapes are to some degree dynamic and experience temporal changes (Hanski 1999b; Jump & Penuelas 2005). In the wetlands project (chapters 2, 3), I investigated these temporal changes in plant species composition in fragmented landscapes. I studied the possible temporal changes of the habitat fragmentation effects in protected fragmented wetlands within the last 10 years (history and present situation were considered), and compared similarity–distance relationships (a measure related to ß-diversity) between two census periods (1995/97- 2005/06).

In the *Arabidopsis* project (chapters 4, 5), we manipulated both the degree of fragmentation and the rate of patch disturbance by creating static and dynamic landscapes. We created both static and dynamic landscapes by either creating patches which were fixed through time or by destroying and regenerating of habitat patches in new locations in the landscape.

Observational study: wetlands project

Chapter 2 We found an increase in compositional similarity of vascular plant species communities over geographic distance within ten years (1995/2005-06) indicating ongoing homogenization within the study region. However, the pattern of increasing homogeneity and decreasing variation in species composition was not uniform across all altitudinal levels and depended on management type as well as on the taxonomic and ecological group studied.

Compositional similarity of vascular-plant communities between sites generally declined with geographical distance, but at the lower altitudes this general trend was no longer present in the second survey. This homogenization of vegetation composition at lower altitudinal levels was mainly due to generalist species whose presence increased across the entire set of 36 sites. Because this homogenization is due to an increased occurrence of common generalist species, it presents a concern for the conservation of these fen habitats that originally hosted a suite of specialised and endangered species. A remarkable feature of our results was that although ß-diversity in species composition among sites has decreased over the past 10 years**,** alpha diversity has actually increased.

Chapter 3 We found that in general, distance-based isolation metrics were better predictors of plant diversity patterns in calcareous fen meadows than area-based metrics. Total vascular plant species density, and specialist density as well as the Shannon and Simpson index were higher in fens with shorter distance to a neighbouring fen patch. Other studies also showed

that inter-patch distance has a strong effect on connectivity between populations (With & King 1999; Tischendorf & Fahrig 2000a; Goodwin & Fahrig 2002).

Our study indicates that the effects of spatial isolation of a habitat patch are stronger for specialist than generalist species. This is because fen specialists are restricted to fen fragments, and are unable to use the intervening habitat matrix. Thus, as well as the number of neighbouring fen patches and the distance to the next fen, the presence of obstacles to dispersal, i.e. the presence of adjoining land-use elements other than grassland vegetation, resulted in strongly declined species densities. The interaction of log distance to nearest fen × number of neighbouring fens within a 1-km buffer zone around a target fen patch also strongly affected habitat specialists. Specialist' species density decreased strongly in isolated fragments with a long distance to the next patch and few immediate neighbours around. For bryophytes, we found less clear relationships between distance-based isolation metrics and species density. However, again obstacles to dispersal caused a decline in bryophyte species richness after ten years.

In general, effects of landscape structure on plant biodiversity was more pronounced in the second survey than in the first, suggesting that lag effects of habitat fragmentation should be considered in conservation planning.

Experimental study: Arabidopsis project

Chapter 4 The experimental treatment patch disturbance (static vs. dynamic) had a dramatic effect on population density. Differences in density in turn caused differences in the survival rate of seedlings to adulthood, with seedlings experiencing much higher death rates in static than in dynamic landscapes. Our second landscape-level treatment, fragmentation (the size and number of suitable patches), had less impact and was often highly variable in its effects on density, survival and biomass among generations. The surprisingly small effects of fragmentation are perhaps because the total area was approximately constant, while in nature

fragmentation is usually linked to a decrease in the total area of suitable habitat (Fahrig 1997). The fact that at least one of our experimental treatments had such a dramatic effect, despite the variation between generations, strengthens our conclusions that patch dynamics are likely to be a potent force in the evolution of annual plant life-histories.

Chapter 5 We observed very strong selection for tall plants in dynamic compared with static landscapes. This occurred in two ways: First, the frequency of the ERECTA mutation declined to around 8 % in dynamic landscapes compared with 44 % in static landscapes. Second, the height of the surviving individuals whether ERECTA or non-ERECTA was greater in dynamic compared with static landscapes. By generation 5, mean plant height in dynamic landscapes was more than twice the value in static landscapes. Interestingly, although there was strong selection for tall plants in dynamic landscapes, there appeared to be much weaker selection for short plants in static landscapes. Thus, the average height in static landscapes is similar to the average height among the original lines.

There was a highly significant decline in seed mass in dynamic landscapes over time, but not in static landscapes. In static landscapes there appears to have been very little selection on measured traits. Particularly, there dose not seem to have been selection for large- seeded genotypes. This is despite the fact that competition was intense in static landscapes with a large number of seedlings failing to survive to adulthood (Chapter 4). There is therefore the intriguing possibility that seed size in static landscapes was selectively neutral (Turnbull et al., 2008). Interestingly, in our landscape experiment, the effect of disturbance regime on plant traits (seed size and plant height) was much more important than the area of the habitat patches.

CONCLUSION

The observational study revealed that conservation planning should take into account the lag

effect of habitat fragmentation on plant species diversity, because populations and metapopulations may respond with a time lag to the structure of the landscape (Tilman et al., 1994; Hanski 1998; Ovaskainen & Haski 2002). In addition, spatial arrangement of the habitat patches as well as the interaction effects of spatial parameters are important concerns in landscape design. Isolation measures (e.g. increasing distance, presence of barriers, reducing number of neighbouring habitats) have more serious consequences for regional plant species survival when occurring in combination than when occurring separately.

The experimental study revealed that disturbance regime was more important than area of the habitat patches, both at the scale of the landscape and at the scale of individual plants. Thus, patch dynamics are likely to be a potent force in the evolution of annual plant life-histories. Theses facts strengthen our conclusions that temporal change and landscape dynamics have an important role and should be taken into account in landscape scales studies on biodiversity.

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Summary

Summary

This study focused on the effects of landscape patterns (habitat fragmentation and spatial structure) and landscape dynamics (changes in landscape patterns through time) on plant communities, using both observational and experimental approaches, at different scales from natural landscapes to experimental microcosms.

Chapter 2

Similarity in species composition between sites typically decreases with geographical distance due to a decrease in environmental similarity or due to dispersal limitation. We studied the decay of similarity with distance between plant communities (a measure related to ß-diversity) among 36 species-rich, pre-alpine fen meadows distributed across north-eastern Switzerland both in 1995/97 and again in 2005/06. We tested whether the pattern of similarity decay over geographical distance differed 1) between altitudinal levels (800–1000, 1000–1200, 1200–1400 m a.s.l.), 2) between management regimes (mown vs. grazed), 3) between taxonomic groups (vascular plants vs. bryophytes), and 4) between specialist and generalist plant species. In addition, 5) we tested whether patterns showed temporal consistency by comparing similarity–distance relationships between the two census periods. Geographical distance between pairs of sites was defined as edge-to-edge distance and similarity in species composition was measured with the Jaccard index using species presence/absence data. These distance data were analyzed with Mantel tests and multiple regression models. Compositional similarity among vascular-plant communities decayed significantly with geographical distance only at higher altitudes (in 1995/97: 1000–1200m and 1200–1400m, in 2005/06: only at 1200–1400m), but not at the lowest altitude. For grazed fens, the rate of decay was significant in 1995/97, but not anymore in 2005/06. Furthermore, decay of similarity over geographical distance in generalist vascular plants and bryophytes was significant in 1995/97, but not in 2005/06. These results indicate that ß-diversity in species composition among sites

has decreased over the past 10 years. Because this homogenization is due to an increased occurrence of common generalist species, it presents a concern for the conservation of these fen habitats that originally host a suite of specialised and endangered species.

Chapter 3

This study investigated how landscape and fen characteristics (patch size, distance between fens, number of neighbouring fens, management type), surrounding habitat types, and potential landscape permeability may influence vascular plant and

bryophyte species diversity, and assesses temporal change of the spatial effects in these protected wetlands within the last 10 years. We studied an archipelago of 36 species-rich calcareous fen meadows identifiable by their typical *Carex davalliana* vegetation type in the Swiss pre-Alps (800–1400m a.s.l.) for two time steps (1995/97, 2005/06). Our randomly chosen fens were all traditionally managed, but differed in the type of management (mown vs. grazed). Inter-patch distance had the most consistent and strongest influence on plant diversity in our fen meadows whereas area-based metrics were less important at least in the first census. The effects of spatial isolation of a habitat patch were stronger for specialist than generalist species. Total vascular plant species density, and specialist density as well as the Shannon and Simpson index were higher in fens with shorter distance to a neighbouring patch. Specialist vascular plant species density was positively affected by the number of habitat neighbours and negatively affected by the presence of potential dispersal barrier, i.e. by vegetation types other than open grassland, around the focal patches, whereas generalist vascular plants were not influenced by dispersal obstacles. For bryophytes that are good dispersers, we found a less clear relationship between landscape metrics and species density except for obstacles to dispersal, which negatively affected bryophyte species density at the second census in 2005/06. In general, effects of landscape structure on plant biodiversity, i.e. especially on specialist species density, were more pronounced at the time of the second

census in 2005/06, suggesting lag effects of habitat fragmentation that should therefore be considered in conservation planning.

Chapter 4

We studied multi-generational experimental metapopulations of *Arabidopsis thaliana*. We created artificial landscapes with islands of suitable habitat embedded in an unsuitable matrix to simulate habitats in nature. We manipulated both the degree of fragmentation by using four patch sizes, and the rate of patch disturbance by using two different patch disturbance regimes, such that patches either remain in place (static) or were destroyed and re-created in new locations (dynamic). The number of seedlings in each patch, the number of surviving adults, and the total biomass were measured in each generation. These variables were analysed using linear mixed-effects models, considering landscape identity as a random effect. Patch disturbance had a strong effect on population density with much higher numbers of seedlings in static than in dynamic landscapes. These differences in density in turn caused differences in the survival of seedlings to adulthood, with seedlings experiencing much higher death rates in static than in dynamic landscapes. We also found a positive relationship between total biomass and density of the surviving adults. The landscape fragmentation treatment (the size and number of suitable habitat patches) had less impact and was often highly variable in its effect among generations. The fact that at least one of our experimental landscape treatments had such a dramatic effect strengthens our conclusions that patch dynamics are likely to be a potent force in the evolution of annual plant life-histories.

Chapter 5

We employed experimental metapopulations to investigate how landscape characteristics may influence plant morphological traits associated with dispersal ability in fragmented landscapes. We selected a population of 19 recombinant inbred lines (RILs) of *Arabidopsis*

thaliana, varying in their seed mass and height. Ten of the selected lines carry the ERECTA mutation: these lines are expected to achieve poorer spatial dispersal of their seeds. We manipulated both the degree of fragmentation (by using four patch sizes, and the rate of patch disturbance, by using two different patch disturbance regimes (static vs. dynamic). Dynamic landscapes are characterized by continual patch destruction and regeneration, while patches in static landscapes remain in place for several generations. We measured the effects of five generations of selection on plant traits (height, number of branches and seed mass) in the 24 manipulated landscapes. To exclude the confounding effects of density and to confirm whether genuine selection had occurred, seeds sampled from generation 5 plants were grown under standardized conditions (a single plant per pot). We found a strong effect of disturbance regime on average plant height. There was strong selection for taller plants in dynamic compared with static landscapes. This resulted in both changes in the frequency of the ERECTA mutation and changes in the height of the surviving individuals, both ERECTA and non-ERECTA in static vs. dynamic landscapes. The absolute height in static landscapes was similar to the average height among the original lines while plants in dynamic landscapes were much taller. Plants in dynamic landscapes also had smaller seeds after 5 generations of selection, while the average seed mass in static landscapes was similar to the original lines. Thus, in the static landscapes there appears to have been very little selection on measured traits while in dynamic landscapes there was rapid evolution toward higher dispersal ability.

Zusammenfassung

Zusammenfassung

Diese Arbeit befasst sich mit den Auswirkungen von Landschaftsmustern (Habitatfragmentierung und räumliche Struktur) und Landschaftsdynamik (zeitliche Veränderungen von Landschaftsmustern) auf Pflanzengemeinschaften. Es wurden sowohl beobachtende als auch experimentelle Ansätze auf verschiedenen räumlichen Skalen - von natürlichen Landschaften bis zu experimentellen Mikrokosmen -verwendet.

Kapitel 2

Die Ähnlichkeit von Standorten in ihrer Artzusammensetzung nimmt üblicherweise aufgrund der abnehmenden Ähnlichkeit der Umwelt oder wegen Verbreitungslimitierung einzelner Arten mit der geographischen Distanz ab. Wir untersuchten diesen Ähnlichkeitsabfall mit der Distanz zwischen Pflanzengemeinschaften (ein sich auf die ß-Diversität beziehendes Mass) an Hand von 36 artenreichen, voralpinen Flachmoorwiesen in der Nordostschweiz in den Jahren 1995/97 und nochmals 2005/2006. Wir untersuchten, ob das Muster des Ähnlichkeitsabfalls über die geographische Distanz von 1) der Höhenlage (800–1000, 1000–1200, 1200–1400 m ü.d.M.), 2) der Bewirtschaftungsform (Mahd vs. Beweidung), und 3) der taxonomischen Gruppe (Gefässpflanzen vs. Bryophyten) abhängt, und sich 4) zwischen Spezialisten und Generalisten unterscheidet. Zusätzlich untersuchten wir durch den Vergleich der Ähnlichkeits-Distanz Beziehung zwischen den beiden Untersuchungszeiträumen, ob 5) die Muster zeitlich beständig sind. Die geographische Distanz zwischen Standortpaaren wurde als Rand-zu-Rand Distanz zwischen Flachmoorflächen definiert und die Ähnlichkeit der Artzusammensetzung wurde mittels Jaccard-Index, basierend auf der An- oder Abwesenheit von Arten, gemessen.

Diese Distanzdaten wurden mit Mantel-Tests und Multipler Regression ausgewertet. Die Ähnlichkeit der Artzusammensetzung von Gefässpflanzen-Gemeinschaften nahm nur in den

oberen Höhenstufen signifikant mit der Distanz ab (1995/97: 1000–1200m und 1200–1400m; 2005/06: nur 1200–1400m), jedoch nicht in der untersten Höhenstufe. Für beweidete Flachmoorwiesen war die Abnahme signifikant für 1995/97, nicht aber für 2005/06. Gleichermassen war der Ähnlichkeitsabfall mit der geographischen Distanz für Generalisten und Bryophyten nur 1995/97, nicht jedoch 2005/2006 signifikant. Diese Resultate deuten an, dass die ß-Diversität bezüglich der Artzusammensetzung von Standorten im Lauf der vergangenen 10 Jahre vor allem in den unteren Höhenstufen abgenommen hat. Weil diese Homogenisierung auf verstärktes Auftreten von häufigen Generalisten zurückzuführen ist, zeigt sie, dass die Sorge um den Schutz dieser Flachmoore, welche ursprünglich zahlreiche spezialisierte und gefährdete Arten beherbergten, berechtigt ist.

Kapitel 3

In dieser Studie untersuchten wir wie Landschafts- und Flachmooreigenschaften (Fläche, Distanz zwischen Flachmooren, Anzahl benachbarter Flachmoore, Bewirtschaftungsform), die umgebenden Habitattypen und die potenzielle Durchlässigkeit der Landschaft die Artenvielfalt von Gefässpflanzen und Bryophyten beeinflussen können, und massen die zeitliche Änderung der räumlichen Effekte in diesen geschützten Feuchtgebieten während der letzten 10 Jahre. Wir untersuchten einen Archipel von 36 artenreichen, kalkgründigen, durch die typische von *Carex davalliana* geprägte Vegetation erkennbare Flachmoorwiesen in den Schweizer Voralpen (800–1400m ü.d.M.) zu zwei Zeitpunkten (1995/97, 2005/06). Unsere zufällig gewählten Wiesen wurden alle traditionell bewirtschaftet, jedoch mit unterschiedlichen Bewirtschaftungsformen (Mahd vs. Beweidung). Die Distanz zu anderen Flachmoorhabitaten hatte in unseren Flachmoorwiesen den einheitlichsten und stärksten Einfluss auf die Artenvielfalt, während sich flächenbasierte Grössen als weniger wichtig erwiesen, zumindest im ersten Untersuchungszeitraum. Die räumliche Isolation von Habitatflächen wirkte sich bei Spezialisten stärker aus als bei Generalisten. Sowohl die Dichte

aller Gefässpflanzen und Bryophyten als auch der Shannon- und Simpson-Index waren in Flachmooren mit geringer Distanz zu einer Nachbarfläche erhöht. Die Dichte spezialisierter Gefässpflanzen wurde durch die Anzahl benachbarter Flächen positiv aber durch die Anwesenheit potentieller Verbreitungsgrenzen, wie z.B. andere Vegetationstypen als Wiesen in Umgebung der zentralen Flächen, negativ beeinflusst. Generalistische Gefässpflanzen wurden hingegen nicht durch Verbreitungshindernisse beeinflusst. Für die sich leicht verbreitenden Bryophyten beobachteten wir einen weniger klaren Zusammenhang zwischen Landschaftsmassen und Artdichte, mit Ausnahme von Verbreitungshindernissen, die im zweiten Untersuchungszeitraum 2005/06 einen negativen Einfluss auf die Artdichte der Bryophyten hatten. Im Allgemeinen waren die Effekte der Landschaftstruktur auf die pflanzliche Biodiversität, insbesondere auf die Artdichte der Spezialisten, während des zweiten Untersuchungszeitraums 2005/2006 ausgeprägter. Dies deutet auf eine zeitliche Verzögerung der Wirkung von Habitatfragmentierung hin, welche deshalb bei der Planung von Schutzmassnahmen berücksichtigt werden sollte.

Kapitel 4

Wir untersuchten die experimentellen Multigenerations-Metapopulationen von *Arabidopsis thaliana*. Um natürliche Habitate nachzuahmen, erschufen wir künstliche Landschaften, mit Inseln von günstigem, jedoch in eine unwirtliche Matrix eingebettetem, Habitat. Wir veränderten den Grad der Habitatfragmentierung mittels vier Grössen von Teilflächen und die Häufigkeit von Störungen durch Anwendung zweier verschiedener Störungsregime. Habitatflächen verblieben entweder an derselben Stelle (statisch) oder wurden jeweils zerstört und an einem neuen Ort wieder erstellt (dynamisch). Dann wurden für jede Generation die Anzahl Sämlinge pro Fläche, die Anzahl überlebender Adulte und die Gesamtbiomasse gemessen. Diese Variablen wurden mit linearen mixed-effects Modellen untersucht, wobei die Landschaftsidentität als zufälliger Effekt behandelt wurde. Die Störung der Flächen hatte

einen starken Einfluss auf die Populationsdichte: die Anzahl Sämlinge war in den statischen Landschaften viel grösser als in den dynamischen. Diese Dichteunterschiede führten ihrerseits zu unterschiedlichen Überlebenschancen der Sämlinge zu ausgewachsenen Pflanzen. Sämlinge in statischen Landschaften wiesen viel höhere Sterblichkeitsraten auf als jene in dynamischen Landschaften. Des Weiteren beobachteten wir einen positiven Zusammenhang zwischen der Gesamtbiomasse und der Dichte der überlebenden Adulten. Die Landschaftsfragmentierung (Grösse und Anzahl geeigneter Habitatflächen) hatte einen geringeren Einfluss, der sich auch unterschiedlich auf die einzelnen Generationen auswirkte. Die Tatsache, dass zumindest eine unserer experimentellen Landschaftsbehandlungen einen so dramatischen Einfluss hatte, bekräftigt unsere Schlussfolgerung, dass die Dynamik von Habitatsflächen vermutlich eine treibende Kraft für die Evolution der Life-Histories annueller Pflanzen ist.

Kapitel 5

Wir verwendeten experimentelle Metapopulationen, um zu untersuchen wie Landschaftseigenschaften die morphologischen Eigenschaften von Pflanzen (welche in fragmentierten Landschaften stark mit der Verbreitungsfähigkeit zusammenhängen) beeinflussen können. Wir wählten eine Population von 19 rekombinanten, durch Inzucht erzeugten Linien (RILs) von *Arabidopsis thaliana aus*, welche sich in Samengewicht und Wuchshöhe unterschieden. Zehn der gewählten Linien tragen die sogenannte ERECTA Mutation. Für diese Linien erwarteten wir eine schwächere räumliche Samenverbreitung. Wir veränderten den Grad der Habitatfragmentierung durch vier Grössen von Teilflächen und die Häufigkeit von Störungen durch Anwendung zweier verschiedener Störungsregime (statisch vs. Dynamisch. S. Kapitel 4). Dynamische Landschaften waren durch ständige Flächenzerstörung und -regeneration geprägt, während statische Landschaften über mehrere Generationen an fester Stelle blieben. Wir massen die Effekte von Selektion auf

Pflanzeneigenschaften (Höhe, Anzahl Verzweigungen und Samengewicht) in den 24 veränderten Landschaften über 5 Generationen. Um verfälschende Dichteeffekte auszuschliessen und um zu erhärten, dass echte Selektion stattgefunden hat, wurden die von 5 Generationen gesammelten Samen unter standardisierten Bedingungen angezogen (eine Pflanze pro Topf). Wir beobachteten einen starken Einfluss des Störungsregimes auf die durchschnittliche Höhe der Pflanzen. In den dynamischen Landschaften wirkte eine im Vergleich zu statischen Landschaften starke Selektion für höher gewachsene Pflanzen. Dies führte zu unterschiedlicher Häufigkeit der ERECTA Mutation und zu veränderten Wuchshöhen der überlebenden Individuen, sowohl mit als auch ohne ERECTA Mutation, in statischen gegenüber dynamischen Landschaften. Die absolute Wuchshöhe in statischen Landschaften war ähnlich wie die durchschnittliche Höhe der ursprünglichen Linien, während Pflanzen in dynamischen Landschaften viel höher wuchsen. Pflanzen in dynamischen Landschaften wiesen nach 5 Generationen Selektion auch kleinere Samen auf, während das mittlere Samengewicht in statischen Landschaften ähnlich dem der ursprünglichen Linien war. Es scheint also, dass in den statischen Landschaften sehr wenig Selektion auf die gemessenen Eigenschaften aufgetreten ist, während in dynamischen Landschaften eine rasche Evolution hin zu höherer Verbreitungsfähigkeit auftrat.

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